Bull. Fac. Life Env. Sci. Shimane Univ., 17：9－21, September 30, 2012

Effect of forage quality and protein level in the diet on growth performance and nitrogen balance in lambs after weaning

Tsutomu FUJIHARA[#] and Toshiyoshi ICHINOHE

Abstract Three trials have been done to investigate growth performance, nutrient digestibility, N balance and plasma levels of Insulin and some metabolites in lambs weaned at 45 days after birth and fed diets consisted with concentrates and roughages of different protein level (high-protein; HCP and low-protein; LCP), and also compared the food value of ammoniated barley straw, ammoniated rice straw or the mixed hay (predominant Italian ryegrass hay) as basal feed for growing stage in early weaned lambs. Total 14 lambs (4, trial I; 6, trial II; 4, trial III) were adopted after drinking colostrum, and thereafter they were reared by feeding a milk replacer supplemented with casein to meet the protein requirement of animals during their growth. They were weaned at 45 days, and were raised in metabolism cages (Trial I & III) or individual pen (Trial II) through the entire experimental period. There were no clear differences of feed intake of lambs between HCP and LCP groups. As a whole, average DG during 0 to 20-24 weeks did not clearly reflect the difference of dietary protein level, though the values tended to high in HCP group than that in LCP group. Through the experimental period, CP digestibility tended to high in HCP group than in LCP group, and contrarily urinary N excretion tended to high in HCP group than LCP group. Consequently, there was no clear difference in retained N between both groups, and the ration of retained N to absorbed N was resulted in higher in LCP group than in HCP group. As basal feed for growing stage in weaned lambs, the utilization of mixed hay (predominant Italian ryegrass) could be superior as compared with ammoniated barley and/or rice straws.

Introduction

In adult ruminant animals, it is well known that the dietary protein ingested is mostly degraded into peptide, amino acids and ammonia by microbes, and then, using them the microbes synthesize their own protein, so called microbial protein, in the rumen (Obara, 1987). Therefore, the protein to be digested in lower gut of ruminants is mainly the microbial protein synthesized in the rumen. In other side, even after weaning, the process of protein digestion described above is not complete in young ruminants, because their rumen will be under the developing condition (Lane and Abrecht, 1991). So, during the period for weaning, a drastic change in quality or quantity of feed, *e. g.*, from liquid type to solid type, will have great effects on their performance, in particular physiological situation. Then, it has been formerly reported that there are remarkable changes in plasma levels of growth hormone, Insulin, insulin-like growth factor 1 (IGF-1), glucose and free fatty acids (NEFA) along with growing after early weaning, *i. e.*, through changes in absorptive systems from mono-gastric type to ruminant type through a developed rumen, and these changes will continue along with 2-3 weeks after weaning (Lane and Abrecht, 1991). According to Breier et al., (1988) Insulin and IGF-1 in the plasma of calf decreased markedly after weaning, and they had suggested this phenomenon probably induced by the change in the system of nutrient absorption related to the development

[#] Present address: PCC, Science City of Munoz, Nueva Ecija, Philippines 3123

of the rumen, *i. e*., from mono-gastric to ruminant type. Therefore, it can be easily thought to that a change in quality and/or quantity of feed, in particular the dietary protein obviously has an effect on the growth, nitrogen (N) balance, and plasma levels of hormones and metabolites in lamb.

In the present study, the growth performance, nutrient digestibility, N balance and plasma levels of Insulin and some metabolites were investigated using lambs weaned at 45 days after birth and fed diet consisted with concentrates and roughages of different protein level, and also compared the food value of *ammoniated barley straw, ammoniated rice straw* and *mixed hay* (*predominant Italian ryegrass hay*) as basal feed for growing stage in early weaned lambs.

MATERIALS AND METHODS

Animals and feeding management

Trial I: Four crossbred (Suffolk X Japanese Corriedale) twin lambs (female: nos, 5 & 6) and 2 lambs (male and female: nos. 2 & 7), were adopted in the present experiment just after drinking colostrum, and thereafter they were reared by feeding a milk replacer (MR) supplemented with casein to meet the protein requirement of animals during their growth. This artificial nursing was to be possible for avoiding the effect of a difference in dairy performance of dams on growth rate of lambs after birth (Fujii, 1996). The feeding level until weaning was calculated to meet the requirements of protein and energy based on the NRC standard (1985). The frequency of bottle feeding was 8 times a day (1/8 of ration) in the first week after birth, and was 6 times a day (1/6 of ration) in the 2nd week after birth. Then, the frequency of feeding was 4 times a day (1/4 of ration) in the 3rd and 4th weeks after birth, and later on, it was 3 times a day (1/3 of ration) until weaning (45 days after birth). The experimental animals were fed in individual pens until 3rd week after birth, and then, they were kept in the metabolism cages and freely offered ammoniated barley straw (basal diet), rolled barley and commercial concentrate pellets for calf until weaning. Water and salt licks containing trace elements were accessible at all times. After weaning, they were fed ammoniated barley straw as basal diet, rolled-barley and soybean protein, and a half of daily ration at 09:00 and 17:00, respectively.

Trial II: Three crossbred (Suffolk X Japanese Corriedale) twin lambs (2 males: nos. 1 & 6) and 4 female lambs (nos. 2. 3, 4, & 5), were adopted in the present experiment just after drinking colostrums, and thereafter they were reared by feeding a milk replacer (MR) supplemented with casein to meet the protein requirement of animals during their growth. They were fed in individual pens until 3rd day after birth, and then, they were kept in the metabolism cages and from 2 weeks after weaning, freely offered timothy hay, Alfalfa hay-cube and rolled barley until weaning. On 45th day after birth (weaning), they were transferred from metabolism cages to individual pens. They were fed on the diet consist of ammoniated rice straw (basal diet), soybean meal and mixed molasses. The other procedures were as described above (Trial I).

Trial III: Four crossbred (Suffolk X Japanese Corriedale) twin (male: nos. 2 & 3) and 2 lambs (male: nos. 1 & 4), were adopted in the present experiment just after drinking colostrums, and thereafter they were kept in the metabolism cages. They were reared by feeding a milk replacer (MR) supplemented with casein to meet the protein requirement of animals during their growth as same as in Trial I. They were castrated before weaning within one month after birth. After weaning on 45 days, the animals transferred from metabolism cages to individual pens, and were freely offered the mixed hay (predominant Italian-ryegrass hay) (basal diet), soybean meal and mixed molasses. The other procedures were as described above (Trials I & II).

Experimental procedure

Trial I: At the 45 days after birth, the animals were adopted into two groups, *i. e*. low protein (LCP) (nos. lamb 6 & 7) and high protein (HCP) (nos. 2 & 5) groups; LCP, 1.2 times of maintenance level; HCP, 2.0 times of maintenance level in NRC standard (1985). The daily ration was composed with ammonia-treated barley straw as basal diet, and rolled barley and soybean-protein as additives (see Table 1). The feeding level was weekly calculated based on the body weight measured once a week (see Table 3). One half of the daily ration was offered at 09:00 hour and another half at 17:00 hour. The orts were collected daily just before morning and evening feed to check daily feed intake during the experiment.

Table 1. Nutritional composition of ingredients consisted the daily ration (% DM)

Ingredient	DM[#]	OM	CP	EE
Trial I				
Ammoniated barley straw	89.71	95.62	9.31	2.50
Rolled barley	87.88	97.64	13.25	2.60
Soybean protein	92.98	95.33	84.50	0.82
Trial II				
Ammoniated rice straw	89.20	80.70	8.90	1.60
Mixed molasses	84.50	88.60	11.90	1.20
Soybean meal	91.00	92.1	34.60	1.70
Trial III				
Mixed hay*	88.70	88.00	8.40	1.60
Mixed molasses	84.50	88.60	11.90	1.20
Soybean meal	91.00	92.10	34.60	1.70

[#] DM: Dry matter, OM: Organic matter, CP: Crude protein, EE: Ethel extract.
* Predominant Italian ryegrass hay.

The animals were weighed at birth, and later on once a week before morning feed during the experiment. On the 0, 1, 2, 3, 8, 12, 16, and 20 weeks after weaning, the orts, feces and urine were collected daily just before morning feed during the consecutive 5-days to measure the feed intake, digestibility of nutrients and nitrogen (N) balance in lambs. The orts and fecal sample were dried for 48 hours in forced air drier at 55 degree centigrade. Urine was collected daily in bottle added about 100 ml of 10% (v/v) sulfuric acid to reduce pH (below 3.0) for preventing an N loss. The blood sample was also collected on −1, 0, 1, 2, 3, 12, 20 weeks after weaning to measure blood glucose level and the levels of insulin and non-esterified fatty acid (NEFA) in plasma of lambs. On the blood sampling day, the feed remained in a manger was removed on 12 hours before first sampling to avoid some effect of intake of any food on the blood levels of hormone and/or metabolites. About 10 ml of jugular blood was sampled through heparinized syringe just before morning feed, and 1, 2, 3, 4, 5, 6 and 7 hours after first sampling on the final day of digestion trials and they were immediately centrifuged in 15 minutes (1,630xg) to separate plasma. The plasma was stored under −40 degree-centigrade until analyses.

Trial II: At the 45 days after birth, the animals were adopted into two groups, *i. e.* low protein (LCP) (nos. lamb 2, 4 & 6) and high protein (HCP) (nos. 1, 3 & 5) groups. The daily ration was composed with ammonia-treated rice straw as basal diet, and rolled barley (1 week after weaning), molasses (2[nd] to 22[nd] weeks after weaning) and soybean meal as additives (see Table 1). On the 0, 1, 2, 3, 10, 14, 18, and 22 weeks after weaning, they were moved to metabolism cages, and feces and urine were collected daily just before morning feed during the consecutive 5-days to measure feed intake, digestibility of nutrients and nitrogen (N) balance in lambs. On the 4, 11, 15, 19 and 23 weeks after weaning, the passage rate of digesta was measured using Yb (Mader et al., 1984) and Co-EDTA (Uden et al., 1980) as markers for solid and liquid phases, respectively. The other procedures except blood sampling were as described above (Trial I).

Trial III: At the 45 days after birth, the animals were adopted into two groups, *i. e.* low protein (LCP) (nos. lamb 1 & 3) and high protein (HCP) (nos. 2 & 4) groups. They were transferred to individual pen from metabolism cages, and fed the daily ration composed with mixed hay (predominantly Italian ryegrass) as basal diet, molasses mixed with rice bran and soybean meal. On the 1, 2, 3, 4, 5 and 6 months after weaning, they were moved to metabolism cages, and feces and urine were collected daily just before morning feed during the consecutive 5-days to measure feed intake, digestibility of nutrients and nitrogen (N) balance in lambs. The blood sample was also collected on 0, 1, 2, 3, 4, 8, 12, 16, 20 and 24 weeks after weaning to measure blood glucose level and the levels of insulin, total protein and urea N in plasma of lambs. About 10 ml of jugular blood was sampled through heparinized syringe just before morning feed, and 0, 2, 5 and 7 hours after first sampling on the final day of digestion trials. The other procedures except blood sampling were as described above (Trial I).

Analytical procedures

Trial I: Nitrogen in the diet, orts, feces and urine was analyzed by the Kjeldahl method, and the contents of crude fat, crude fibre and crude ash in the diet, orts and feces were determined according to AOAC method (Hoitz, 1960). The blood glucose, insulin and NEFA in plasma were analyzed using commercial test-reagents (Wako Chemical Co., Osaka, Japan).

Trial II: Yb and Co in the fecal samples was determined using Inductively Coupled plasma Emission Spectroscopy

(ICPS-2000, Shimadzu Co., Kyoto, Japan). The other procedures except about measurements of hormone and metabolites in plasma were as described above (Trial I).

Trial III: The procedure for preparing urine for analysis was the same as described earlier (Fujihara et al., 2007), and the analysis of urinary allantoin was done according to the method of Young and Conway (1942). The microbial protein synthesized in the rumen was calculated by the equation proposed by Chen and Gomes (1992). The other procedures including about measurements of hormone and metabolites in plasma were as described above (Trial I).

Statistical analysis

Tests for significance of differences between the two groups (HCP and LCP groups) were undertaken by *t*-test.

RESULTS AND DISCUSSION

Feed intake and growth performance

Trial I: Table 2 shows feed intake in lambs summarized weekly after weaning. There was fairly refusal of roughage and concentrate until 3rd week after weaning, and on 8, 12, 16 and 20 weeks after weaning the refusal of concentrate decreased, although roughage refusal was still quite more. This would indicate the rumen was still under developing stage in the lambs at that time. As a whole, the DM intake gradually increased along with an increase of body weight, but in the late stage of experiment the DM intake decreased in lamb 2 (20th week) and lamb 7 (12th & 16th weeks), and so the growth rate also declined slightly. The DM intake per metabolic body weight in lambs 2 and 7 was the highest in 2nd week, and in lambs 5 and 6 the values were highest on 8th week after weaning, and then, their body weight was about 15kg (head). There were no differences of DM intake of lambs between LCP and HCP groups.

The daily gain (DG) in each week after weaning was also shown Table 3. The body weight at birth was relatively more heavy in single lamb than in twin lambs, and so the values (4.10 and 5.00kg) of single (nos. 2 and 7) was almost two times to that (2.75 and 1.95kg) of twin (nos. 5 and 6). The differences in birth weight of lambs have had really effects on growth performance in the present experiment. The growth of lambs 2 (HCP) and 7 (LCP) was faithfully after birth, although there was some differences in DG along with aging of animals. Before weaning the difference of body weight in two lambs (nos. 2 and 7) was mostly same along with the lapse of time after birth, and then after weaning their body weight was almost equal at 8th week, and later on the growth rate of lamb 2 (HCP) was superior to that of lamb 7 (LCP) until final stage of

Table 2 Feed intake of lambs after weaning (g/BW$^{0.75}$/d)

Week	Trial I+		Trial II		Trial III	
	HCP	LCP	HCP	LCP	HCP	LCP
0	53.1± 2.9[#]	46.6± 9.6	33.1±15.3	22.1± 3.4	−	−
1	65.3± 4.2	59.5±10.9	52.5±11.0	56.5± 0.7	−	−
2	71.9±12.9	64,7± 9.8	69.6± 2.7	62.7± 0.3	−	−
3	70.5± 5.9	70.5± 3.8	69.5± 4.1	74.3±10.8	−	−
4	−	−	−	−	69.4±2.8	73.1±4.8
8	71.9± 8.4	70.9±11.8	−	−	74.9±0.7	79.8±3.3
10	−	−	61.3± 4.1	60.1± 6.0	−	−
12	62.3± 0.1	58.2± 9.3	−	−	76.7±0.2[a]	84.4±0.8[b]
14	−	−	68.7± 2.0	60.1± 6.0	−	−
16	57.9± 6.0	54.3±10.6	−	−	79.2±4.5	81.6±8.1
18	−	−	70.4± 5.2	72.6± 8.9	−	−
20	53.4± 5.8	59.9±10.7	−	−	79.8±5.4	89.9±4.6
22	−	−	74.3± 4.1	78.7± 1.2	−	−
24	−	−	−	−	75.5±0.9	75.1±5.5

[+] DM intake

[#] Mean ± S.D (n=2∼3 lambs X 7 days)

[a, b] : Values in the same row with different superscripts differ significantly (P<0.05)

experiment. This phenomenon would be due to an effect of gender in both animals rather than dietary protein level, because the growth rate of male lamb is ordinarily thought to be superior to that of female lamb in young stage (NRC, 1985). There was also obvious increase of feed intake in lamb 2 than in lamb 7 described above. In the twin lambs (nos. 5 and 6), there was also no clear effect of dietary protein level on their growth rate, and after 12th week the feed intake of lamb 6 (LCP) was more than that of lamb 5 (HCP) resulting an increase of DG in the former than in the latter. As a whole, there would be no clear effect of dietary protein level on the growth rate of lambs after weaning at 45 days after birth. In the present study, feeding level was calculated to meet 200, 250 and 300g DG at 10, 20 and 30kg body weight, respectively. However, the average DG of two animals at 10, 20 and 30kg body weight was 271, 291 and 229g; 229, 251 and 144g in HCP and LCP groups, respectively. Consequently, average DG during 0 to 20th week did not clearly reflect the difference of dietary protein level, although the value tended to high in HCP group than that in LCP group.

Trial II: The daily gain (DG) in each week after weaning is also shown in Table 3. The average DG during the experimental period was 68.8±16.0 and 55.3±14.0g/d in HCP and LCP groups, respectively. In comparison of DG in each animal between twins, there was a difference in DG between 2 groups, and also between male and female, *i. e*., daily feed intake ($g/d/BW^{0.75}$) was also more in male than in female as described generally. The feeding level was set up to achieve 200g as DG according to NRC standard (1985), however the DG attained in this trial was 68.8 and 55.3g in HCP and LCP groups, respectively. This would be attributed to lower energy intake in lambs during the period, because they have mostly consumed the daily ration from 10th week to final day after weaning but nutrient digestibility were relatively low in both groups as compared that described generally.

Trial III: The daily gain (DG) of each animal during the period are also shown in the Table 3. The feed intakes indicated as per metabolic body weight ($g/kg^{0.75}/d$) are shown in Table 2. The average DG during the period was 107.2±60.8 and 84.5±44.0 in HCP and LCP groups, respectively. The total body weight gain (kg) during the experimental period was 18.4-21.4 and 14.8-18.0 in HCP and LCP groups, respectively. Feed intakes tended to high in LCP group than in HCP group, and the difference at 3rd month after weaning was significant ($P<0.05$) statistically. The DG tended to high in HCP group than in LCP group, although the feed intake per metabolic body weight tended to lower in the former than in the latter. This would be due to the difference in dietary protein level. As shown above, average DG in both groups was 107.2 (HCP) and 84.5g (LCP) and these values were quite lower than that recommended by AFRC standard (1993). This could be due to a relatively low ruminal synchrony of dietary energy and protein in the present experiment.

Nutrient digestibility and N balance

Trial I: Table 4 shows nutrient digestibility in lambs at 0, 1, 2, 3, 8, 12, 16 and 20 weeks after weaning, although in lamb No. 7 at 0 week after weaning the samples could not collect due to slushy feces. The DM and organic matter digestibility was higher at earlier stage (0 to 2nd week), and later the values became smaller (almost 77%) in all the animals. In CP digestibility there was same trend as in DM (OM) digestibility during the experiment. There was no clear difference in digestibility of nutrient between

Table 3 The monthly changes in daily gain of lambs (g)

Month	Trial I		Trial II		Trial III	
	HCP	LCP	HCP	LCP	HCP	LCP
1	253.6±186.9#	225.0± 75.5	500.0±447.2	358.3±462.1	93.3±56.6	76.7±42.4
2	232.1±121.6	228.5±154.1	650.0±627.4	546.7±537.3	136.7±42.4	130.0±14.1
3	210.6±131.5	175.0± 81.4	533.3±580.5	550.0±560.0	188.3±118	145.0±54.2
4	158.6±196.2	110.3±143.9	600.0±620.8	533.3±561.4	111.7±77.8	51.7±96.6
5	60.8± 77.1	132.1±153.1	433.3±596.0	583.3±663.1	110.0±42.4	70.0±33.0
6	42.5± 60.1	57.0± 80.6	133.3±250.0	–	3.7±10.1	33.3± 4.7

Mean±S.D of 2~3 lambs x 4 (weeks).

Table 4 Monthly changes of Nutrient digestibility in lambs (%)

Month	Trial	DM		OM		CP		EE		TDN	
		HCP	LCP	HCP	LCP	HCP	LCP	HCP	LCP	HCP	LCP
1	I	78.0±0.6[#]	76.9±2.5	78.6±0.1	77.9±2.7	86.5±1.3	80.7±0.4	74.7±6.3	79.0±4.8	75.4±0.5	73.8±1.5[b]
	II	72.9±1.7	69.2±2.3	78.2±1.5	75.0±1.7	77.4±1.7	70.1±3.5	72.5±5.9	77.3±2.0	56.0±1.3	53.5±2.0
	III	76.7±3.2	69.1±0.6	77.2±3.2	69.4±0.4	77.5±3.3[a]	53.4±5.2[b]	50.0±3.0	53.1±15.3	–	–
2	I	77.5±0.1	75.9±2.1	76.9±2.3	92.3±2.1	92.3±2.1	78.8±2.1	86.1±3.4	82.3±8.8	74.1±0.1	72.2±2.0
	II	75.4±2.2	71.2±0.2	78.7±2.1	74.7±0.5	78.9±3.7	67.2±5.2	58.6±12.2	46.1±33.9	60.6±16.0	57.7±1.5
	III	70.6±2.9	65.0±3.0	71.6±2.1	65.9±3.1	77.9±0.2[a]	66.2±3.3[b]	44.5±3.7	49.7±2.1	–	–
3	I	73.1±0.9	74.2±0.4	74.9±0.8	75.5±0.2	85.5±0.1	75.4±1.6	81.0±9.8	79.5±9.9	69.3±1.3	70.3±0.5
	II	69.6±1.3	66.6±1.5	73.2±1.3	73.1±5.3	72.6±2.7	62.1±4.9	46.3±3.0	53.6±22.9	57.0±1.0	50.9±7.0
	III	72.0±0.4	69.0±6.3	72.8±0.9	72.5±7.9	80.8±4.0	72.5±7.9	46.1±5.9	47.4±4.9	–	–
4	I	75.3±2.3	76.1±1.5	77.7±0.9	77.5±0.8	86.2±1.8	79.1±1.1	71.3±15.0	72.5±1.0	71.8±0.2	72.6±1.8
	II	66.4±1.1	66.6±1.5	70.0±1.4	69.5±6.4	68.3±2.6	60.3±6.6	45.1±0.9	57.7±18.4	55.0±0.8	52.5±0.9
	III	72.8±5.3	64.6±1.6	73.9±6.0	66.0±1.6	71.9±9.5	53.6±8.3	51.9±22.3	40.1±0.3	–	–
5	I	78.7±1.3	77.8±3.2	80.5±1.6	78.8±2.9	85.1±2.3	79.9±3.0	80.9±9.1	78.6±3.5	73.2±0.6	74.1±3.3
	II	67.0±0.9	64.8±4.5	71.4±0.9	69.9±4.0	67.4±3.0	57.7±6.7	81.9±4.4	91.2±6.7	53.8±0.7	51.3±3.5
	III	71.7±0.6	68.6±2.1	72.7±1.0	69.2±1.9	71.4±1.2	58.6±5.4	48.4±10.6	42.6±12.3	–	–
6	I	–	–	–	–	–	–	–	–	–	–
	II	–	–	–	–	–	–	–	–	–	–
	III	70.5±0.4[a]	65.2±1.2[b]	71.0±0.4[a]	65.2±1.2[b]	65.1±2.2[a]	48.0±1.8[b]	48.9±12.0	42.4±9.7	–	–

[#] Mean±S.D of 2-3 lambs X 5-7days. [a, b] : Values in the same row with different superscripts differ significantly ($P<0.05$)

HCP and LCP groups of animals. Generally in early weaned lamb, it is well known that they cannot utilize solid feed enough to meet their requirement for growth (Lane et al., 1986). Then if they have ingested the solid feed enough to meet that they needs, but the digestibility of them would be generally low due to an immature of the rumen. Funaba *et al*. (1994) also reported in calves reared by milk replacer that the digestibility was relatively low just after weaning, and then it was gradually higher along with their aging after weaning (Funaba *et al*., 1994). In the present experiment, there was relatively high digestibility at early stage after weaning, and this finding due to low intake of roughage and contrary high intake of concentrate. During 0 to 2^{nd} week after weaning the digestibility of nutrient were almost the same in both groups of HCP and LCP. The values after 3^{rd} week however were obviously higher in HCP group than in LCP group. The digestible organic-matter intake (DOMI) was the highest (about $60g/kg^{0.75}/d$) at 2^{nd} and 8^{th} week in lamb nos. 2 and 7, and 5 and 6, respectively. The value of metabolizable energy (ME) recommended by NRC (1985) was 7.12, 13.81, 16.74 and17.16 (MJ/d/head) at 10, 20, 30 and 40kg body weight, respectively. The figures calculated in the present experiment were about 3-7 (MJ/d/head) lesser than the NRC recommendation during the period (0 to 20 week after weaning) in all the animals, *i. e*., about 60% of planned.

Table 5 shows the nitrogen balance in lambs during the experiment after weaning. Nitrogen intake in animals was about 1.5 times higher in HCP group than in LCP group, but the amount consumed did not reach to the amounts to meet DG planned at initiation of study in both groups because the animals did not consume all the diet offered. Fecal N output was almost the same in both groups, although urinary nitrogen excretion was obviously high in HCP group than in LCP group. As a result, absorbed N increased along with aging of animal in both groups, and average values during the experiment (0 to 20 weeks) was 23.5-33.6g and 7.5-21.7g in HCP and LCP groups, respectively. The retained N also increased slightly after weaning, and their maximum value was observed at 8^{th} week in lamb nos. 2, 5 and 7, and in lamb No. 6, the highest value was observed at 16^{th} week, and later on it tended to decrease slightly in all the animals. As a whole, the retained N tended to increase in HCP group than in LCP group. As a result,

Table 5 Nitrogen balance of lambs fed HCP and LCP diet (g/d^{+} & g/BW$^{0.75}$/d)

Month	Trial	Intake		Fecal N		Urinary N		Retained N		RN/AN(%)$^{\#}$	
		HCP	LCP	HCP	LCP	HCP	LCP	HCP	LCP	HCP	LCP
1	I	32.9±5.9*	21.9±2.0	4.0±1.2	3.8±0.4	13.5±1.7	5.0±1.7	15.1±2.9	12.9±0.2	52.8±2.4	72.2±6.4
	II	2.3±0.2	2.1±0.5	0.5±0.0	0.6±0.1	0.9±1.1	0.6±0.2	0.8±0.2	0.9±0.3	46.4±7.1	62.2±1.7
	III	1.8±0.0	1.4±0.1	0.4±0.1^{a}	0.7±0.0^{b}	0.9±0.0^{b}	0.3±0.0	0.6±0.0	0.4±0.0	40.0±0.0^{a}	56.7±4.2^{b}
2	I	42.4±4.3	25.1±1.0	4.5±0.7	4.6±0.3	19.0±5.1	6.3±1.6	18.9±1.5	14.2±0.3	50.3±8.8	69.5±5.7
	II	1.8±0.1	1.3±0.3	0.4±0.1	0.4±0.0	0.7±0.1	0.4±0.3	0.8±0.2	0.4±0.4	55.0±9.2	54.9±28
	III	2.2±0.0^{b}	1.6±0.0^{a}	0.5±0.0	0.5±0.1	1.1±0.0^{b}	0.4±0.1^{a}	0.6±0.0	0.6±0.1	36.8±1.4	59.8±12
3	I	45.1±9.4	24.5±0.6	5.6±1.6	4.9±0.2	22.1±7.4	6.0±3.2	17.3±0.7	13.6±2.8	44.7±7.3	66.8±19
	II	1.9±0.1	1.4±0.2	0.5±0.0	0.5±0.1	0.5±0.2	0.3±0.2	0.9±0.1	0.6±0.3	66.4±9.2	60.0±29
	III	2.3±0.0^{b}	1.7±0.0^{a}	0.5±0.1	0.5±0.1	1.5±0.2^{b}	0.5±0.2^{a}	0.4±0.1^{a}	0.7±0.1^{b}	22.2±7.3	58.0±11
4	I	45.8±5.8	25.4±0.7	6.0±1.7	4.5±0.4	23.8±7.4	7.7±6.0	16.0±3.3	13.2±5.7	40.8±12	63.4±28
	II	1.8±0.0	1.5±0.1	0.6±0.1	0.6±0.1	0.7±0.2	0.3±0.0	0.5±0.2	0.6±0.1	66.4±12	67.5±4.8
	III	2.1±0.3	1.5±0.1	0.6±0.1	0.7±0.1	1.0±0.2	0.4±0.3	0.5±0.2	0.4±0.1	34.0±5.7	52.0±25
5	I	38.5±4.0	26.5±1.3	4.9±0.1	4.6±1.4	20.5±0.8	9.1±0.2	13.1±4.9	12.6±0.4	38.4±9.8	58.0±1.2
	II	1.8±0.1	1.5±0.1	0.6±0.0	0.6±0.1	0.8±0.2	0.3±0.1	0.5±0.1	0.6±0.1	39.0±13	63.5±3.7
	III	1.8±0.1	1.7±0.2	0.5±0.0^{a}	0.7±0.0^{b}	0.7±0.1	0.4±0.2	0.6±0.0	0.6±0.0	49.0±1.2	58.0±16
6	I	–	–	–	–	–	–	–	–	–	–
	II	–	–	–	–	–	–	–	–	–	–
	III	1.8±0.0^{b}	1.2±0.1	0.6±0.0	0.7±0.1	0.7±0.2	0.2±0.0	0.5±0.1	0.4±0.0	43.8±14	62.0±2.8

$^{+}$Trial I, $^{\#}$ Retained N/Absorbed N(%), * Mean±S. D. of 2–3 lambs X 5–7 days. $^{a, b}$: Values in the same row with different superscripts differ significantly ($P<0.05$)

it could be thought that an ammoniated barley straw will not be useful as basal roughage with no supplement for early weaned lambs.

Trial II: As shown in the Table 4, the digestibility of DM and OM was high at 1 to 10 weeks after weaning in both groups, and there was a rough trend to high in HCP group as compared with those in LCP group. The digestibility of CP also showed similar trend of DM/OM, and the values in HCP group was not lower than that in LCP group during the experimental period. This would be reflected to a high CP intake in HCP group than in LCP group.

The changes in N balance and utilization of dietary N during the experimental period (g/BW$^{0.75}$/d) are shown in the Table 5. The daily N intake tended to high in HCP group than in LCP group, and the faecal N tended to slightly low in the former than in the latter. Urinary N excretion was clearly higher in HCP group than in LCP group. Then, the retained N during the experimental period tended to high in HCP group than in LCP group, and this could be reflected to the difference in growth rate between the both groups of HCP and LCP. The N utilization (retained N/absorbed N), however, tended to slightly high in LCP group than in HCP group. This would be due to physiological saving N absorbed in LCP group, and also in HCP group the energy to up take N into tissues was not enough, and so as a result, an excess N was excreted into urine. Similar result has been reported in growing steers after changed from milk replacer to solid feed after weaning (Lane et al., 1986). Energy intake would not be enough to utilize dietary N, because the refusal of feed was little after 10 weeks in HCP group, although the daily gain tended to higher than that in LCP group. Then, it seems that some supplement as energy source would be needed when ammoniated rice straw is used as a basal feed for early weaned lambs.

Trial III: As shown in the Table 4, DM digestibility during the experiment was 70–76 and 64–69% in HCP and LCP groups, respectively. The highest value was observed at 1 month after weaning in both groups, and also the lowest value was at 6 and 4 months after weaning in HCP and LCP groups, respectively. The difference of the value at 6 month between groups was significant ($P<0.05$) statistically. The digestibility of OM showed almost similar trend with DM during the experiment in both groups. The appar-

Table 6 The changes in passage rate (%/h) and retention time of digesta in alimentary tract of lambs (Trial II)

Weeks	4	11	15	19	23
Roughage in the rumen					
HCP group	3.7±0.5#	3.0± 0.3	2.4±0.1	3.5±0.1	3.3±0.2
LCP group	3.9±0.3	2.9± 0.7	3.1±0.1	3.0±0.0	3.6±0.2
Roughage in lower gut					
HCP group	38.0±5.9	30.0± 9.4	26.0±5.8	36.0±9.0	34.0±1.2
LCP group	35.0±1.4	31.0± 4.6	29.0±3.6	40.5±1.2	37.5±6.0
Liquid phase					
HCP group	4.5±0.4	3.7± 0.0	3.9±0.3	4.6±0.3	4.3±0.4
LCP group	4.1±0.3	3.8± 0.3	4.3±0.1	5.2±0.7	4.8±0.3
Retention time of digesta					
HCP group	30.0±3.3	37.0± 1.1	46.0±0.1	32.0±1.1	34.0±1.1
LCP group	29.1±1.9	40.0±12.2	38.0±0.1	36.0±0.0	31.0±1.7

Mean ± S. D. of 3 lambs.

ent digestibility of CP during the period was 65-80 and 48-72% in HCP and LCP groups, respectively. The lowest value was observed at 6 month after weaning in both groups, and the difference of values between groups at 1, 2 and 6 months after weaning was significant ($P<0.05$) statistically. The digestibility of EE was 44-51 and 40-53% in HCP and LCP groups, respectively. The highest value during the period was observed at 4 and 1 months after weaning in HCP and LCP groups, respectively. The lowest value in EE digestibility was observed at 2 and 4 months in HCP and LCP groups, respectively. The nutrient digestibility tended to high in HCP group as compared to that in LCP group through the experiment. The digestibility of DM, OM and CP tended to high as compared with those of trial II, and this would indicate that the utilization of mixed hay (predominant Italian ryegrass) could be superior as basal diet for growing lambs as compared to ammoniated rice straw.

The result of N balance ($g/BW^{0.75}/d$) is shown in the Table 5. Fecal excretion tended to high in LCP group than in HCP group during the experiment, and on 1 and 5 months the difference was significant ($P<0.05$) statistically. On the other hand, urinary N excretion was clearly high in HCP group as compared to that in LCP group, and on 1, 2 and 3 months the difference between both groups was significant ($P<0.05$) statistically. As a result, retained N was higher in HCP group than in LCP group except the value on 3 month, and this would be reflected on difference of DG in both groups. As a whole, N utilization (retained N/absorbed N) was 37.5 and 57.7% in HCP and LCP

Table 7 Urinary excretion of allantoin and microbial protein synthesis in the rumen of lambs fed HCP and LCP diets (Trial III)

Month	1	2	3	4	5	6
Allantoin excretion ($mmol/BW^{0.75}/d$)						
HCP group	0.40±0.05#	0.68±0.10	0.95±0.32	0.54±0.01	0.46±0.11	0.63±0.18
LCP group	0.32±0.16	0.62±0.17	0.61±0.01	0.54±0.27	0.52±0.04	0.66±0.24
Microbial protein synthesis (g/d/h)						
HCP group	16.7± 2.6	37.4± 5.3	61.8±25.2	37.7± 4.1	33.4± 6.0	49.2±20.2
LCP group	12.8±11.5	33.6± 7.8	38.3± 2.7	33.9±15.3	35.9± 6.0	47.4±22.7

Mean ± S. D. of 2 lambs X 5 days.

groups, respectively, and this phenomenon was similar to that observed in Trial II. During the experimental period, water intake and urine excretion tended to high in HCP group than in LCP group, and then it might reflect on the difference in N balance between both groups.

Passage rate of digesta

The passage rate of ingesta in lambs at each week after weaning in both groups is shown in the Table 6 (Trial II). The passage rate of liquid phase decreased during 4-11 weeks after weaning in both groups, and later on until 19 weeks it increased and it decreased again till 22 weeks. Then, it could be assumed that the rate of passage of liquid phase is probably thought to reach a stable condition at 22 weeks after weaning. The passage rate of solid phase through lower guts (post-rumen) decreased at 4-15 weeks and increased until 19 weeks after weaning, and later on it decreased a little till 22nd week. Then, the passage rate of solid phase is also thought to reach a stable condition at 22 weeks after weaning as well as that of liquid phase. The ruminal passage rate of solid phase was almost the same as that in lower gut in HCP group, however in LCP group it decreased at 4-11 weeks and increased at 19-22 weeks after weaning. There was a negative relationship between the mean retention time in total digestive tract and the passage rate in the rumen. The reticulo-rumen takes about 65% of total digestive tract as volume, and is about 80% of all the stomach. Then, it is easily presumed that the feed ingested could stay long time, and the passage rate in the reticulo-rumen would be thought to one of factors that influences the retention time of digesta in total digestive tract. The change in passage rate of ingesta through the lower gut will be related to the development of the lower gut, however the ratio (as weight) of the abomasums to total stomach will decrease from 49% (at birth) to 11% (as mature) (Ohkubo, 1984). The weight of total stomach will increase with an increase of body weight, but the relative ratio of each part of stomach changes a little after 16 weeks after birth (Ohkubo, 1984). In the present study the passage rate of ingesta through the rumen increased after 15 weeks (150 days after birth), and then the rumen is thought to be developed as mature stage at that time. The rumen contents are thought to move into the abomasums through the omasum with relatively constant rate (Grovum and William, 1973). The factor that control passage rate of contents in the abomasums is thought to be volume of the abomasum and/or physical and chemical properties of ruminal contents. The fact that the passage rate of digesta though lower guts including the abomasums was relatively high, the retention time of digesta in lower guts was surly short (Murphy et al., 1994) and this is not roughly inconsistent with that the digestibility of DM, OM and CP obviously decreased along with an

Table 8 The changes in blood glucose level of lambs fed HCP and LCP diet after weaning (mg/dL)

Week after weaning	Trial I		Trial III	
	HCP	LCP	HCP	LCP
−1	85.4± 2.8[#]	132.0±13.8	−	−
0	94.5±15.4	112.4±26.1	84.7± 9.3	78.2± 2.6
1	97.2±23.0	75.6±20.6	79.3± 9.3	76.1± 8.0
2	90.8±10.4	89.5±12.1	79.3± 1.9	77.7± 4.2
3	91.2±11.8[b]	134.7±15.7[a]	79.6± 2.7	77.0± 0.4
4	−	−	83.0± 3.4	80.8± 3.1
8	−	−	79.8± 2.6	77.8± 4.2
12	−	−	78.8± 3.0	71.2± 3.0
14	74.6±13.7[b]	127.9± 8.8[a]	−	−
16	−	−	79.6± 1.1	71.7± 4.1
20	−	−	77.7± 4.2	71.6± 4.3
22	90.2±18.0	102.8±17.5	−	−
24	−	−	74.3± 1.4	70.6± 5.1

[#] Mean± S. D. of 2 lambs X 0～7hours after morning feed.

[a, b] : Values in the same row with different superscripts differ significantly ($P<0.05$)

aging in ruminants. The changes in passage rate of liquid phase would be also thought to an effect of increase of water intake along with an improvement of the rumen. From these findings, the effect of dietary protein level on the passage rates of digesta through the rumen and/or lower guts, and also the rate of passage of liquid phase after feeding roughage.

Urinary allantoin excretion

Urinary allantoin excretion and microbial protein synthesis in the rumen of lambs of both groups are shown in the Table 7 (Trial III). On 1～3month after weaning the values tended to high in HCP group as compared to those in LCP group, and thereafter the figures were in a range of 0.46～0.66mmol/BW$^{0.75}$/d until end of the experiment in both groups. These were very comparable to that urinary allantoin level of 0.27～0.50mmol/BW$^{0.75}$/d in growing lambs fed fattening diet at 60% level of almost full feeding (Fujihara et al., 1999). There was no clear difference in the values of both groups during the experimental period. In HCP group, the heist value of urinary allantoin was observed on 3 month after feeding, and it was corresponded to the heist values of CP digestibility and of DG at 3 month after weaning. This obviously shows an increase in metabolized protein of microbial protein (MP), and then, this could influence to CP digestibility and DG in the lambs. Then, the MP synthesized calculated by the method of Chen & Gomes (1992) has also shown similar tendency of that in urinary allantoin excretion during the experiment.

The microbial protein synthesized in the rumen tended to high in HCP group than in LCP group through the experimental period, and this would indicate that dietary protein level had influenced on microbial activity in the rumen of lambs.

Changes in blood levels of hormone and metabolites

Trial I: Glucose and insulin levels in the blood of lambs at 37- and 45-day-old are shown in the Table 8. There were some variations in the values at 37th day among animals, although before weaning, all the animals were reared in same condition. Blood glucose level in lambs of LCP group was almost the same until 7 hours after morning feed, and contrarily in HCP group (Lamb nos. 2 and 5) average level was relatively lower than that in LCP group after morning feed, and there was different changing pattern in 2 animals. The plasma insulin level did not change in good relation with the changes of blood glucose after morning feed. At 45th week after birth, the changing pattern in blood glucose was similar in 2 lambs of 2 groups, although average value was slightly higher in LCP group than that in HCP group. The changing pattern of plasma insulin level was not consistent as a whole among animals in both groups.

The levels of blood glucose, insulin and NEFA in plasma after weaning were shown in the Table 9. There was no tendency in the change of blood glucose level in lambs along with the lapse of time after weaning, although there was an individual variation in both groups. The values were

Table 9 The changes of insulin and NEFA concentrations in blood plasma of lambs fed HCP and LCP diet after weaning

Week after weaning	Insulin (μU/dl)				NEFA (mEq/L)	
	Trial I		Trial III		Trial I	
	HCP	LCP	HCP	LCP	HCP	LCP
−1	5.56±1.58[#]	4.43±1.47	−	−		
0	6.23±2.12	5.10±2.45	3.76±1.42	4.43±0.25	0.393±0.186	0.363±0.136
1	5.36±2.11	3.86±1.34	3.21±0.43	3.15±0.57	0.297±0.220	0.482±0.180
2	5.54±2.27	7.14±1.31	2.80±0.94	3.84±1.68	0.281±0.207	0.157±0.112
3	8.37±0.93	5.73±3.21	3.84±0.65	4.83±1.54	0.420±0.092	0.168±0.140
4	−	−	4.74±1.39	5.13±0.86	−	−
8	−	−	4.94±1.27	7.14±1.65	−	−
12	−	−	5.77±0.98	5.65±2.37	−	−
14	3.01±1.11	8.21±2.21	−	−	−	−
22	9.60±1.25	9.24±1.63	−	−	−	−
24	−	−	5.37±1.80	5.25±1.40	−	−

[#] Mean ± S. D. of 2 lambs X 0～7 hours after morning feed.

Table 10 The changes in the concentrations of plasma urea nitrogen and blood total protein of lambs fed HCP and LCP diet (Trial III).

Week after weaning	Urea N		Total protein	
	HCP	LCP	HCP	LCP
0	28.5±0.9[#, a]	14.9±2.0[b]	5.17±0.14	5.47±0.10
1	25.3±0.7[a]	16.2±1.5[b]	5.43±0.33	5.82±1.05
2	22.0±0.4[a]	14.3±1.0[b]	5.16±0.15	5.27±0.13
3	25.7±2.2[a]	13.8±0.9[b]	5.14±0.22	5.40±0.09
4	22.5±0.5[a]	12.4±0.5[b]	5.49±0.16	5.49±0.20
8	25.9±1.6[a]	13.5±0.3[b]	5.68±0.11	5.79±0.17
12	29.3±1.0[a]	18.7±1.8[b]	5.87±0.26	5.80±0.18
16	29.2±1.4[a]	13.8±0.9[b]	5.83±0.11	6.03±0.21
20	28.4±2.9[a]	19.5±1.3[b]	6.02±0.08	6.11±0.12
24	21.0±1.5[a]	15.5±2.0[b]	6.10±0.07	6.12±0.14

[#] Mean ± S. D. of 2 lambs X 4 times.
[a, b] : Values in the same row with different superscripts differ significantly ($P<0.05$)

relatively high, and then it would be assumed that development of the rumen was not enough to digest roughage diet, and so they would have mainly eaten concentrate diet rather than roughage feed. Consequently, the digestive system of them would be as same as that of mono-gastric animals, and so the blood glucose level was quite high as compared to that in ruminants. In the changes of plasma insulin level, there was also similar tendency in lambs with the lapse of time after weaning as that in blood glucose. The plasma NEFA level in lambs also did not change drastically after weaning, although there were some individual variations. These findings probably show that weaning at 45 days after birth could be stress to the lambs, and then, they could not get the feed enough to meet the requirement of their body until 20 weeks after weaning in both groups, and is not different from the results reported previously (2, 3).

Trial III : As shown in the Table 10 (Trial III), plasma urea N was high in HCP group as compared to that in LCP group during the period. In HCP group, the plasma urea N tended to increase during 4–12 weeks after weaning, and then the level tended to decrease until 24 weeks after weaning. On the other hand, the concentration of plasma urea N was almost constant during the experiment except the levels on 12 and 20 weeks after weaning in LCP group. The time course changes after morning feed tended to much higher in HCP group than in LCP group. The average levels during the period in the both groups are slightly lower than that (18～42mg/dl) reported by Blood et al. (1983) and are very comparable to that (8～20mg/dl) reported by Swenson (1990). According to Preston et al., there was a relative high correlation between N intake and blood urea N level in ruminant (Preston et al., 1965), and in this trial, the same correlation was also observed ($R=0.7609$). Lewis et al. reported that plasma N level could be reflected the ammonia production in the rumen (Lewis, 1957), and so, in this trial, it would be thought that ammonia level in the rumen was higher in HCP group than in LCP group. It seems to that rumen function in lams would be satisfied on 12 weeks after weaning, and then, the relatively high level of rumen ammonia in HCP group at 12 weeks after weaning could be due to shortage of energy for incorporating ammonia by microbes in the rumen. As a result, an excess ammonia N was absorbed directly through the rumen wall, and it was converted to urea in the liver to circulate in blood stream.

The changes in blood glucose concentration in lambs of both groups after weaning are shown in Table 9, and the pattern in the changes along with the progress of weeks after weaning was higher in HCP than in LCP group. The highest value was observed at 4 weeks, and then gradually decreased till 12 weeks after weaning in both groups. In general, blood glucose level in ruminants will be normally lower than that in non-ruminants, and the average value is constantly shown as 60mg/dl (Lewis, 1957). In this trial the blood glucose level was usually higher than about 60mg

/dl and this clearly due to age of animals (growing stage).

The concentrations of plasma total protein in lambs after weaning are shown in Table 10, and the time course changes of the values along with the growth was always higher in LCP group than in HCP group during the experiment. On the 4 weeks to 16 weeks the figures increased and thereafter that was almost constant till 24 week after weaning. The plasma level of total protein in sheep is normally 6.0～8.0g/dl in sheep (Fujihara et al., 1999), and then the values in this trial were slightly lower than the values described above. This would be due to low utilization of dietary N in lambs, because the plasma level of urea N was relatively high as compared with other data (Blood, 1983), and it shows a relatively low production rate of microbial protein in the rumen, *i. e*., it will be a just opposite a relatively high level of plasma urea N as described above.

CONCLUSION

In this study, effect of dietary protein level (1.2 : LCP and 2.0 : HCP X maintenance) and various roughages such as ammoniated barely and rice straws and mixed hay used as a basal feed on growth performance and nitrogen metabolism was investigated using early weaned lambs. Daily gain tended to high in HCP group as compared to that in LCP group, but not significantly ($P>0.05$). This would be due to a relatively low ruminal synchrony of dietary energy and protein in the present study, because the roughage used here did not utilize for providing enough energy to meet required in the rumen at that time. As a whole, it seems that some supplement as energy source could be needed when the roughages used here as a basal feed for early weaned lambs.

ACKNOWLEDGEMENTS

We are grateful to Ms T. Tokuda, Mr. T. Hirano and Ms Y. Togawa for their helpful assistance during the course of experiment.

REFERENCES

1) Obara, Y. (1987) Metabolism of nutrients in the rumen: nitrogen compounds. In: Shin Nyuugyuu no Kagaku (Tsuda, T. and Shibata, F. ed.) Nohsan-gyoson-Bunkakyoukai. Tokyo, pp.132-143. (In Japanese).

2) Lane, S. F. and Abrecht, K. A. (1991) Growth and plasma metabolites of lambs weaned to legume pastures at 28 days of age. *Journal of Animal Science*, **69**: 305-317.

3) Breier, B. H., Glickman, P. D. and Bass, J. J. (1988) Concentrations of insulin-like growth factor-I and insulin in the infant calf: ontogeny and influence of altered nutrition. *Journal of Endocrinology*, **119**: 43-50.

4) Fujii, Y. (1996) Effect of dietary protein level on nitrogen balance and the passage rate of digesta in growing lambs. MS thesis, Shimane University. (In Japanese)

5) NRC. (1985) Nutrient Requirements of sheep, 6th ed. National Research Council, National Academy of Sciences, Washington, DC.

6) Mader, T. L., Teeter, R. G. and Horn, G. W. (1984) Comparison of forage labeling techniques for conducting passage rate studies. *Journal of Animal Science*, **58**: 208-212.

7) Uden, P., Colucci, P. E. and VanSoest, P. J. (1980) Investigation of chromium, cerium and cobalt as markers in digesta rate of passage studies. *Journal of the Science of Food and Agriculture* **31**: 625-632.

8) Hoitz, H. (ed.). (1960) Official methods of Analysis, 9th ed. AOAC, Washington, D. C. pp.283-288.

9) Fujihara T, Matsui T, Harumoto T. (1991) Urinary excretion of purine derivatives and blood plasma level of allantoin in sheep and goats during fasting. *Proceedings of the 6th International Symposium on Protein Metabolism and Nutrition*, pp.170-172.

10) Young, E. G. and C. F. Conway. (1942) On the estimation of allantoin by the Rimini-Schryver reaction. *Journal of Biological Chemistry*, **142**: 839-852.

11) Chen, X. B. and Gomes, M. J. (1992) Estimation of microbial protein supply to sheep and cattle based on urinary excretion of purine derivatives - an overview of the technical details. IFRU, Rowett Research Institute, Occasional Publication, pp.1-20.

12) Agricultural and Food Research Council (AFRC). (1993) *Energy and Protein Requirements of Ruminants*. An Advisory Manual prepared by the AFRC Technical Committee on Responses to Nutrients. CAB International, Wallingford.

13) Lane, S. F., Magree, B. H. and Hogue, D. E. (1986) Growth, intekes and metabolic responses of artificially reared lambs weaned at 14d of age. *Journal of Animal Science*, **63**: 2018

14) Funaba, M., Kagiyama, K., Iriki, T. and Abe, M. (1994) Changes in nitrogen balance with age in calves weaned at 5 or 6 weeks of age. *Journal of Animal Science*, **72**: 732-739.

15) Ohkubo, M. (1984) The rumen – Function of the Rumen, Dairy Japan Co., Tokyo.

16) Grovum, W. L. and William, V. J. (1973) Rate of passage of digesta in sheep. 4. Passage of marker through the alimentary tract and the biological relevance of rate-constant derived from the changes in concentration of marker in faeces. *British Journal of Nutrition*, **30**: 231-242.

17) Murphy, T. A., Leorch, S. C. and Smith, F. E. (1994) Effect of feeding high-concentrate diets ar restricted intakes on digestibility and nitrogen metabolism in growing lambs. *Journal of Animal Science*, **72**: 1583-1590.

18) Fujihara, T., M. Iwakuni, D. J. Kyle & E. R. Ørskov, The effect of feeding level and frequency on the microbial protein synthesis in the rumen of sheep. Protein Metabolism and Nutrition (Book of abstracts of the VIIIth Int. Symp. Prot. Meta. Nutr.), p. 54, 1999. (Aberdeen, Scotland, UK, 1st-4th, Sept. 1999).

19) Blood, D. C., Radostits, O. M. and Henderson, J. A. (1983) Veterinary Medicine (6th Ed.) Bailliere Tindall, London.

20) Swenson, M. J. (Ed.) (1990) Duku' Physiology (No. 1), Gakusousha Co., P. 33. (in Japanese: Translated by Tomonori Imamichi et al.)

21) Preston, R. L., Schnakenberg, D. D. and Pfander, W. H. (1965) Protein utilization in ruminants. I. Blood urea nitrogen as affected by protein intake. *Journal of Nutrition,* **86**: 281-290.

22) Lewis, D. (1957) Blood urea concentration in relation to protein utilization in the ruminant. *Journal of Agricultural Science, Cambridge*. **48**: 438-453.