Journal of the Korean Society of Grassland and Forage Science 35(3) : 238~244, 2015
http://dx.doi.org/10.5333/KGFS.2015.35.3.238

ISSN 2287-5824 (Print)
ISSN 2287-5832 (Online)

Research Article

Relationship Between Nutrient Supply to Muscle and Adipose Tissues and Nitrogen Retention in Growing Wethers on Forage Based Diets Fed with Different Forage Sources

Da Hye Kim[1], Toshiyoshi Ichionohe[2], Ki Choon Choi[3], Shinichi Oda[4], Akihiko Hagino[5] and Sang Houn Song[2]*

[1]The United Graduate School of Agricultural Sciences, Tottori University, Tottori, Japan,

[2]Faculty of Life and Environmental Science, Shimane University, Matsue, Japan,

[3]Grassland and Forage Research Center, National Institute of Animal Science, Cheonan, Korea,

[4]Faculty of Agriculture, Iwate University, Morioka, Japan,

[5]Laboratory of Animal Physiology, Graduate School of Agricultural Science, Tohoku University, Aoba, Sendai, Japan

ABSTRACT

Three growing wethers were used to investigate the differences in nitrogen (N) retention, blood plasma metabolite concentration and energy-yielding nutrient supply to muscle and adipose tissue. The wethers were fed one of three diets: timothy hay with concentrate (THD), *Italian ryegrass* with concentrate (IRD), and rice straw with concentrate (RSD) for 11 days. The experimental diets were adjusted to the animals to provide 100 g of daily gain. The triglyceride (TG) concentration of blood plasma in arterial and portal veins was higher with THD and IRD than with RSD. Conversely, the available amount of TG in tissues was higher with IRD. The daily amount of glucose and non-esterified fatty acids (NEFA) supplied to muscle tissue and adipose tissue was numerically higher with THD than IRD or RSD. Although N retention did not differ among the diets, it was numerically higher with THD than with IRD or RSD. The results suggest that the difference in the amount of glucose and NEFA delivered to muscle tissue may reflect the N retention in response to forage based diets.

(**Key words :** Blood plasma flows, Blood plasma metabolites, N balance, Forage based diet, Wether lambs)

Ⅰ. INTRODUCTION

In current feeding systems, the energy requirement is indicated with accuracy based on *in vivo* metabolism studies to access energy requirement for maintenance and production. Moreover, ruminal microbial nitrogen supply to the small intestine has been also evaluated quantitatively to estimate metabolizable protein (MP) supply to ruminants (ARC 1984; AFRC 1993).

Recently Kim et al. (2015) reported that low-quality forage, such as *Italian ryegrass* straw (IR), can be used for a long-term feeding system for growing ruminants. The growing wethers were fed on two types of forage based iso-energetic diet; and both of nitrogen (N) intake and digestible crude protein intake were greater for timothy hay (TH) based diet (THD) feeding as compared to IR based diet (IRD) feeding without significant difference in N retention (% of N intake). Additionally, dressing rate, lean meat weight per empty body weight (EBW), weight proportion of subcutaneous fat and abdominal fat were numerically greater for THD as compared to IRD in hogget production performance of Suffolk wethers which were slaughtered at 18 months of age (Kim et al., 2014). The lack of differences in N retention and carcass traits between the diets may be due to the differences in metabolizable energy intake related digestible organic matter intake (Yan and Agnew, 2004), differences in MP supply originating rumen microbes (Clark et al., 1992), amino acid sparing (Neale, 1971; Neale and Waterlow, 1974), gluconeogenesis from the ruminal propionic acid, and urea recycling into the rumen (Sands and Layton, 2009). However, the effect of factors above mentioned was not cleared in the previous studies (Kim et al., 2014; 2015). The plausible reason for the difference in carcass traits might be due to the difference

* Corresponding author : Sang Houn Song, Faculty of Life and Environmental Science, Shimane University, Matsue, Shimane 690-8504, Japan. Tel: +81-852-32-6594; E-mail: sanghoun_song@life.shimane-u.ac.jp

in amount of nutrients absorbed though the gastrointestinal tract (GIT) or delivered to the liver, rate and extant of nutrients processing in the hepatic tissue, which in turn, resulted in the difference in amount of available nutrients for the development and growth of adipocytes or muscle cells.

To elucidate our hypothesis, there is a need to determine the difference of nutrients flux in sheep fed with different type of forage diets. The objective of this experiment was to determine the effect of feeding forage based diet on blood plasma nutrients flow to muscle tissue and adipose tissue in relevant to N balance determination in growing wethers.

Ⅱ. MATERIALS AND METHODS

The animal experiment of this study was conducted at Shimane University from May to October 2014. Use and animal care procedures were approved by Animal Care Committee of Shimane University (Permit Number: MA26-23).

1. Animals and diets

Three spring born (January to February 2014) Suffolk wethers, initial mean body weight (BW) of 27.3 ± 2.3 kg, were used in this study. The animals were allocated to three dietary treatments at the age of 6 to 7 months. First cut TH, IR and rice straw (RS) were used as basal forages with commercial concentrate in this study (Table 1). The animals were kept individually in metabolism crates throughout the experimental period, and fed THD, IRD or RS with commercial concentrate RSD in a one-way layout design. The forages were allowed at 2% BW (dry matter basis) and amount of concentrate allowance was set at 40% of forage intake (as fed basis) of each animal. The diets were designed to satisfy metabolizable energy requirements for 100 g daily gain with a provision of 5% safety margin based on the estimation equations by the AFRC (1993). The animals were fed two equal sized meals at 09:00 and 21:00 hours throughout the experiment.

The animals were surgically prepared under general anesthesia for forming a skin loop enclosing the right carotid artery and for locating indwelling catheters in the mesenteric vein and the hepatic portal vein at 3 months before the start of the experiment. Surgical preparation, post-surgical care and management were conducted in accordance with the guideline of the animal use regulation of Shimane University and also with the ‘Guide for the Care and Use of Agricultural Animals in Agricultural research and Teaching’ (Federation of Animal Science Societies, 1999).

2. Experimental procedures

The animals were housed in an environment controlled room with air temperature being 23 ± 1℃ with free access to water throughout the experimental period. The experiment was carried out in three periods of 11 days, first 7 days for adaptation to the diet, 3 days for metabolism trials and the last one day for blood collections.

During the metabolism trials, daily excretion of feces and urine were recorded for 3 consecutive days. Urine was collected into the container containing 50 mL of 10% (v/v)

Table 1. Chemical composition of the diets used in the feeding trial

Item	Timothy hay	*Italian ryegrass* straw	Rice straw	Concentrate
Organic matter	92.5	95.3	94.8	93.1
Crude protein	7.4	4.7	4.8	22.0
Ether extract	2.0	1.2	1.2	4.5
Neutral detergent fiber	66.0	66.7	67.3	24.8
Acid detergent lignin	6.3	3.8	6.4	1.2
Non-fiber carbohydrates[†]	17.1	22.7	22.2	41.8

Values are dry matter basis (%)

[†] Calculated as 100 − (crude ash + crude protein + ether extract + neutral detergent fiber).

H_2SO_4 to keep urine pH below 3. Representative samples of feeds, orts, feces and urine were obtained daily during the total collection period. On d-11 of the experiment, continuous infusion of para-aminohippuric acid (PAH) solution (1% w/v) was conducted via the mesenteric vein as described by Ortigues et al. (1994). The blood samples at portal vein and artery were simultaneously collected into 10 mL heparinized syringes at every 60 min after morning feeding. Total 12 sets of blood samples were immediately analyzed for hematocrit value. Whole blood samples were centrifuged for 15 min at 4℃, and the blood plasma samples were stored at −21℃ until further analysis.

3. Chemical analysis

The samples of feeds, orts and feces were dried in a forced air oven at 60℃ for more than 48 h. Air dried samples were ground to pass through a 1 mm screen. The N in feeds and feces samples was determined by AOAC method. Blood plasma concentrations of glucose, non-esterified fatty acids (NEFA) and triglyceride (TG) was determined by commercial kits (Glucose C-test Wako, NEFA C-test, TG E-test, Wako Pure Chemical Industries, Osaka, Japan) and PAH concentration of blood plasma samples were determined by the method of Huntington (1982).

4. Calculations

Blood plasma flow rate was calculated using an indicator-dilution technique previously described by Katz and Bergman (1969). One-compartmental pharmaco kinetics model (Oriuchi et al., 1995) was introduced to estimate the rate constant of nutrients absorption from the GIT and first stage nutrients elimination in the hepatic tissue. According to the graphical shape of the change in nutrients concentration of arterial blood plasma (glucose, NEFA and TG) postprandial time, analytical equation was generated based on the pharmacokinetics model. In this model, change in specific nutrients concentrations of arterial blood plasma post feeding (t, hr) can be described as:

$$C_{nutr} = (C \times k_a/(k_a - k_d)) \times (e^{-kd \times t} - e^{-ka \times t})$$

where C_{nutr}, nutrient concentration of arterial blood plasma; C, scale parameter; k_a, rate constant of nutrients absorption though the small intestine (per h) and kd, rate constant of nutrients disappearance at the hepatic tissue by the metabolism of first passage effect (per h). In this model, assumptions, $k_a \neq k_d$ and $k_a > k_d$, were introduced for estimating the parameter relating nutrients absorption, hepatic tissue metabolism and supply to the body tissue. All curves fitted to estimate the parameters were performed using commercial software generated by Daniel G. (Curve Expert 1.4, http://curveexpert.net). The amount of bioavailable nutrients delivered to the tissues the weight of muscle and adipose tissue were calculated using allometric equations listed in by ARC (1980).

5. Statistical analysis

Effects were deemed to be statistically significant when P<0.05, and tendencies were considered to exist when 0.05<P<0.15. Data were analyzed by one-way ANOVA and differences between the means were separated using Duncan's multiple range test. All statistical procedures were performed using SPSS 12.0 (SPSS, 2006).

Ⅲ. RESULTS AND DISCUSSION

1. Blood metabolites

Blood metabolites were shown in Table 2. Blood plasma concentrations of all items investigated were higher for arterial blood than for portal vein blood except that of TG in IRD. Blood plasma concentration of glucose at arterial and portal vein did not differ significantly between the diets. Sletmoen et al. (2000) has suggested that plasma glucose concentration was increased by supplementing with ruminal degradable protein. However, other studies have shown no effect of protein supplementation on blood glucose concentration in ruminants (Krysl et al., 1987; Cheema et al., 1991), and our result agreed with their results. The concentrations of NEFA and TG at arterial of portal blood were not different among the diets.

2. Plasma flows and net flux of nutrients

The EBW, estimated portal plasma flow rate, absorption

Table 2. Blood plasma concentration of glucose, NEFA and triglyceride at arterial and portal vein in growing wether lambs fed forage based diets

	Diet[†]			SEM	*P*-value
	THD	IRD	RSD		
Glucose concentration (mg/dL)					
Arterial	80.2	73.9	72.0	3.79	0.18
Portal	79.1	72.6	70.3	3.74	0.61
NEFA concentration (μEq/L)					
Arterial	258.0	252.3	274.4	2.53	0.57
Portal	139.4	184.8	217.7	3.65	0.19
Triglyceride concentration (mg/dL)					
Arterial	17.2	17.5	11.3	2.53	0.14
Portal	16.9	18.7	11.8	3.11	0.16

[†] THD, Timothy hay with concentrate diet (n=3); IRD, *Italian ryegrass* straw with concentrate diet (n=3); RSD, Rice straw with concentrate diet (n=3)
The results are mean value of concentration of diurnal blood plasma.
SEM, standard error of the mean.
Means within a row with different superscripts differ (P<0.05).

rate constant of nutrients from the GIT and available nutrient circulations were shown in Table 3. The EBW and portal plasma flow rate (L/min) did not differ among the diets. The k_a (fraction/h) variable of glucose and NEFA did not differ among the diets, and the k_a for TG was significantly lower for THD than for IRD or RSD (P=0.01). Additionally, available amount of glucose and NEFA were estimated to be higher for THD than for IRD or RSD, but statistical differences were not detected between the diets. The available amount of TG was significantly higher for IRD than for THD (P=0.04). The difference of basal forages might have resulted the changes in nutrients absorption rate constant and portal plasma flow rate.

Table 3. Empty body weight, portal plasma flow, Absorption rate constant of nutrients and available nutrient circulation in growing wether lambs fed forage based diets

	Diet[†]			SEM	*P*-value
	THD	IRD	RSD		
EBW (kg) [‡]	19.9	20.5	19.9	0.90	0.65
Portal plasma flow (L/min)	1.2	1.3	1.2	0.08	0.39
k_a (/h) [§]					
Glucose	0.04	0.02	0.3	0.006	0.17
NEFA	0.003	0.002	0.002	0.001	0.32
Triglyceride	0.002^{b}	0.004^{a}	0.004^{a}	0.000	0.01
Available amount (g/d) [¶]					
Glucose	1181.6	846.4	822.6	185.62	0.23
NEFA	32.5	22.1	22.4	6.48	0.31
Triglyceride	11.5^{b}	25.4^{a}	19.2^{ab}	2.58	0.04

[†] THD, Timothy hay with concentrate diet (n=3); IRD, *Italian ryegrass* straw with concentrate diet (n=3); RSD, Rice straw with concentrate diet (n=3)
[‡] EBW, Empty body weight; live weight × 0.75 (Kim et al., 2014)
[§] K_a, rate constant of nutrients absorption at the small intestine (fraction/h)
[¶] Available amount was calculated as portal plasma flow (L/d) × concentration of blood plasma (g/L) × k_a × 24
SEM, standard error of the mean.
Means within a row with different superscripts differ (P<0.05).

3. The proration of nutrition in the body

The daily amount of bioavailable nutrients supply to muscle tissue and adipose tissue in growing wethers were shown in Table 4. Bioavailable glucose and NEFA supplied to both of tissues were higher for THD than for IRD or RSD, however, they did not differ among the diets. In addition, the amount of TG showed no significant differences among the diets. As shown in our results, supplement of bio-available nutrients in forage diets showed no significantly different in muscle and adipose tissue of growing wether lambs. However, little is known that the roles of bioavailable nutrients in the concentration of blood plasma were investigated in the *in-vivo* model because of its very complex process.

4. Effect of forage based diets on N balance

The N balance in growing wethers fed with three forage based diets was shown in Table 5. The N intake was not significant differences among the diets. However, fecal N excretion tended to be higher for IRD than the THD or RSD. The N digestibility was higher for THD than for IRD and RSD, it accounted for 71.4, 50.5 and 57.3%, respectively. However, urinary N excretion was higher for THD than IRD and RSD. The N retention and proration of N intake were higher for THD than IRD and RSD. However, there were no significant differences among the diets. N retention increased with an increase in apparent digestible N intake, which was also reported in other sheep breeds (Sarraseca et al., 1998; Lobley et al., 2000; Marini et al., 2004;

Table 4. The bioavailable nutrients flow in muscle tissue and adipose tissue

	Diet[†]			SEM	*P*-value
	THD	IRD	RSD		
Glucose					
Muscle tissue (g/d)[‡]	303.5	217.3	212.3	48.98	0.25
Adipose tissue (g/d)[‡]	258.0	185.2	177.5	38.15	0.20
NEFA					
Muscle tissue (g/d)	8.3	5.6	5.7	1.63	0.29
Adipose tissue (g/d)	7.1	5.0	4.9	1.50	0.34
Triglyceride					
Muscle tissue (g/d)	3.0	8.1	4.9	1.04	0.34
Adipose tissue (g/d)	2.5	7.1	4.2	1.06	0.34

[†] THD, Timothy hay with concentrate diet (n=3); IRD, *Italian ryegrass* straw with concentrate diet (n=3); RSD, Rice straw with concentrate diet (n=3)
[‡] Muscle tissue, $\log_{10}$ Protein mass = $-0.6451+0.8955\times\log_{10}$EBW; Adipose tissue, $\log_{10}$fat mass = $-1.918+1.821\times\log_{10}$EBW (ARC, 1980)
SEM, standard error of the mean.
Means within a row with different superscripts differ (P<0.05).

Table 5. Effect of forage based diets on nitrogen (N) balance in wether lambs

	Diet[†]			SEM	*P*-value
	THD	IRD	RSD		
N intake (g/d)	14.2	13.5	12.4	0.53	0.14
Fecal N excretion (g/d)	4.0	6.7	5.3	0.64	0.06
Urinary N excretion (g/d)	5.3	3.6	3.9	0.56	0.17
N retention (g/d)	4.9	3.2	3.2	0.07	0.30
(% of N intake)	34.1	23.5	25.8	4.03	0.23
(% of absorbed N)	47.4	47.1	45.0	4.69	0.93

[†] THD, Timothy hay with concentrate diet (n=3); IRD, *Italian ryegrass* straw with concentrate diet (n=3); RSD, Rice straw with concentrate diet (n=3)
SEM, standard error of the mean.
Means within a row with different superscripts differ (P<0.05).

Kamalzadeh and Shabani, 2007). In addition, the ratio of N retention to apparent digestible N intake with an increase in apparent digestible N intake increased in 40~50 kg Suffolk wether sheep (Sarraseca et al., 1998).

Ⅳ. CONCLUSION

In conclusion, amount of bioavailable glucose supplement was exhibited no significant differences among the diets. Our results showed that bio-available glucose supplement was higher for muscle tissue than adipose tissue in THD. This reason was thought to be closely related to N retention (g/d or % of N intake) which was higher for THD than for IRD or RSD.

Ⅴ. ACKNOWLEDGEMENT

This study was carried out with the support of “Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ008502032014)”, by Rural Development Administration, Republic of Korea.

Ⅵ. REFERENCES

Agricultural and Food Research Council (AFRC). 1993. Energy and protein requirements of ruminants. An advisory manual prepared by the AFRC Technical Committee on Response to Nutrients. CAB International, Wallingford. pp. 21-31, 91-106.

Association of Official Analytical Chemists (AOAC). 1984. Official method of analysis of the AOAC, 14th edn. AOAC. Arlington, VA.

Agriculture Research Council (ARC). 1980. The nutrient requirement of ruminant livestock, Commonwealth Agricultural Bureaux, Farnham Royal, Slough. pp. 134-158.

Agriculture Research Council (ARC). 1984. The nutrient requirements of ruminant livestock. Commonwealth Agricultural Bureaux, Farnham Royal, Slough. Supplement No.1. pp. 4-11.

Cheema, A.U., Galyean, M.L., Caton, J.S. and Freeman, A.S. 1991. Influence of protein levels and naloxone on ruminal fermentation, serum hormones, and metabolites in lambs fed oat hay or barley straw. Small Ruminant Research. 5:47-55.

Clark, J.H., Klusmeyer, T.H. and Cameron, M.R. 1992. Microbial protein synthesis and flows of nitrogen fractions to the duodenum of dairy cows. Journal of Dairy Science. 75:2304-2323.

Federation of Animal Science Societies. 1999. Guide for the care and use of agricultural animals in agricultural research and teaching. 1st Ed. Federation of Animal Science Societies, Savoy, Ilinoy.

Huntington, G.B. 1982. Portal blood flow and net absorption of ammonia-nitrogen, urea-nitrogen, and glucose in nonlactating Holstein cows. Journal of Dairy Science. 65:1155-1162.

Kamalzadeh, A. and Shabani, A. 2007. Maintenance and growth require-ments for energy and nitrogen of Baluchi Sheep. International Journal of Biological Macromolecules. 9:535-539.

Katz, M.L. and Bergman, E.N. 1969. Simultaneous measurements of hepatic and portal venous blood flow in the sheep and dog. American Journal of Physiology. 216:946-952.

Kim, D.H., Choi, K.C., Ichinohe, T. and Song, S.H. 2014. Evaluation of hogget production of Suffolk fed with forage based diets. Japanese Journal of Sheep Science. 51:9-14.

Kim, D.H., Choi, K.C., Song, S.H. and Ichinohe, T. 2015. Effects of grass forage species and long-term period of low quality forage diet feeding on growth performance, nutrient utilization and microbial nitrogen yield in growing wether lambs. Animal Science Journal (in press).

Krysl, L.J., Branine, M.E., Galyean, M.L., Estell, R.E. and Hoefler, W.C. 1987. Influence of cottonseed meal supplementation on voluntary intake, ruminal and cecal fermentation, digesta kinetics, and serum insulin and growth hormone in mature ewes fed prairie hay. Journal of animal science. 64:1178-1188.

Lobley, G.E., Bremmer, D.M. and Zuur, G. 2000. Effects of diet quality onurea fates in sheep as assessed by refined, non-invasive [$^{15}N^{15}N$] ureakinetics. British Journal of Nutrition. 84:459-468.

Marini, J.C., Klein, J.D., Sands, J.M. and Van Amburgh M.E. 2004. Effect of nitrogen intake on nitrogen recycling and urea transporter abundance in lambs. Journal of Animal Science. 82:1157-1164.

Neale, R.J. and Waterlow, J.C. 1974. The metabolism of ^{14}C-labelled essential amino acids given by intragastric or intravenous infusion to rats on normal and protein-free diets. British Journal of Nutrition. 32:11-25.

Neale, R.J. 1791. Adaptation of amino-acid metabolism in pro-teindepleted rats. Nature New Biology. 231: 117-118.

Oriuch, N., Tokunaga, M., Suzuki, H., Inoue, T., Ohnishi, Y., Inoue, T. and Endo, K. 1995. Unilateral effective renal plasma flow measurement using one-compartment analysis of 99mTc-MAG3 and gamma-camera renography. Nihon Igaku Hoshasen Gakkai Zasshi. 55:757-762.

Ortigues, I., Durand, D. and Lefaivre, J. 1994. Use of para-amino hippuric acid to measure blood flows through portaldrainedviscera, liver and hindquarters in sheep. The Journal of Agricultural Science. 122:299-308.

Sands, J.M. and Layton, H.E. 2009. The physiology of urinary concentration: an update. Seminars in Nephrology. 29:178-95.

Sarraseca, A., Milne, E., Metcalf, M.J. and Lobley, G.E. 1998. Urea recycling in sheep: effects of intake. British Journal of Nutrition. 79:79-88.

Sletmoen-Olson, K.E., Caton, J.S., Kirsch, J.D., Olson, K.C., Reynolds, L.P. 2000. Undegraded intake protein supplementation. II. Effects on blood plasma hormone and metabolite concentrations in periparturient beef cows fed low-quality hay during gestation and lactation. Journal of animal science. 78:456-463.

Yan, T. and Agnew, R.E. 2004. Prediction of metabolisable energy concentrations from nutrient digestibility and chemical composition in grass silages offered to sheep at maintenance. Animal Feed Science and Technology. 117:197-213.

(Received July 20, 2015 / Revised September 6, 2015 / Accepted September 7, 2015)