Journal of Integrated Field Science

Vol. 14

March. 2017

Field Science Center
Graduate School of Agricultural Science
Tohoku University

Ⓒ copyright 2017 by Field Science Center, Graduate School of Agricultural Science, Tohoku University

Journal of Integrated Field Science (JIFS)

Office : Graduate School of Agricultural Science,
Tohoku University,
468-1, Aoba, Aramaki, Sendai,
Miyagi, 980-0845, Japan

Editorial Board :
Yoshihisa Suyama (Japan)
Randy A. Dahlgren (USA)
Joji Iisaka (Canada)
Qi Li (China)
Attila Ombodi (Hungary)
Lilik Budi Prasetyo (Indonesia)
Steven Mark Rutter (UK)
Myung Gyu Lee (Korea)
Akihiro Kijima (Japan)
Makoto Osada (Japan)
Yutaka Nakai (Japan)
Masanori Saito (Japan)
Kenji Seiwa (Japan)
Shin-ichiro Ogura(Japan)
Kiyohide Morita (Japan)
Chinatsu Yonezawa (Japan)

Printed by Meirin-sha Company, Ltd., Sendai, Japan

Contents

Oral Sessions

Poster Sessions

The Growth of Four Tree Species Seedlings on Soil and Decayed Wood of *Pinus densiflora*

Yu FUKASAWA*

***Laboratory of Forest Ecology, Graduate School of Agricultural Science, Tohoku University, Naruko-onsen, Osaki, Miyagi 989-6711, Japan**
Tel: +81 229 84 7397 Fax: +81 229 84 6490

Corresponding Author: Yu Fukasawa
e-mail: fukasawayuu@gmail.com

Keywords: coarse woody debris; dead wood; nitrogen; regeneration; soil microbes

Received 1 September 2016; accepted 30 January 2017

Abstract

Decayed wood provides regeneration microsites for a variety of tree seedlings, whereas the mechanisms of species-specific preferences for decayed wood, their decay types, and soil are poorly known. To evaluate the effects of chemical and biological characteristics of the substrates on seedling preference, I investigated the biotic and abiotic effects of different substrates on the above- and belowground growth of seedlings of 4 tree species commonly occurring in post-Pine Wilt Disease (PWD) forests. Seedlings were grown in microcosms including autoclaved or non-autoclaved substrates (3 types: white-rotted wood, brown-rotted wood, and soil) for 4 months. After retrieval, the above- and belowground growth of the seedlings was measured. *Pinus densiflora* seedlings experienced greater growth in nons-autoclaved rather than autoclaved substrates; however, growth was affected to a lesser extent by the differences in substrates. In contrast, *Cryptomeria japonica* seedlings experienced greater growth in soil than in woods; however, growth was not affected by autoclaving. Aboveground growth of *Clethra barbinervis* seedlings was greater in soil and autoclaved brown-rotted wood than in other wood substrates. The growth of *Eurya japonica* seedlings was greater in soil than in woods. Seedlings of the 3 species, excluding *C. barbinervis*, showed plasticity in the shoot/root ratio against substrate difference. The relative importance of biotic and abiotic factors for the above- and belowground growth of seedlings differ among tree species, which may partly explain species-dependent preferences for different microsites on the forest floor when regenerating in post-PWD forests.

Introduction

Microsite heterogeneity plays an important role in plant population dynamics and species diversity maintenance in forest ecosystems (Harmon and Franklin 1989; Nakashizuka 2001). Different tree species favor specific microsites for seedling regeneration, reflecting their life-history traits associated with physiological and morphological responses to abiotic and biotic environmental conditions (Leck and Outred 2008). Allocation to above- and belowground growth is one of the most conspicuous traits characterizing the initial responses of seedlings to their environments (Kohyama and Grubb 1994; Doi et al. 2008). Thus, it is important to determine allocations of seedlings to above- and belowground growth for understanding their preferences pertaining to forest microsites.

Among the microsites on forest floors, coarse woody debris (CWD) provides important refuges for seedling establishment in various forest ecosystems (Fukasawa 2012). Although CWD generally contains relatively lower concentrations of nutrients compared with soil (Goodman and Trofymow 1998; Baier et al. 2006), it can contain greater concentrations of nutrients compared with soil in some cases (Takahashi et al. 2000; Fukasawa 2015a). Furthermore, the nature of microbial communities inhabiting CWD, particularly the low level of soil-borne pathogens, often contributes to good growth performances of pathogen-susceptible tree seedlings (Cheng and Igarashi 1987; Cheng 1989; Takahashi 1991; O'Hanlon-Manners and Kotanen 2004). However, the extent to which the chemical and biotic properties of CWD contribute to seedling growth is unclear. A couple of studies have recently reported that the decay type of wood, which reflects the wood decay abilities of fungal communities that inhabit CWD, strongly affects seedling establishment on CWD (Bače et al. 2012; Fukasawa 2012). This type has been traditionally categorized into 3 types: 1) white-rot, 2) brown-rot, and 3) soft-rot determined by the chemical composition, physical structure, and outward appearance of wood (Eaton and Hale 1993). Bače et al. (2012) reported that seedlings of *Picea abies* preferentially regenerate on the logs where white-rot fungi dominate than on the logs where brown-rot fungi dominate. On the other hand, Fukasawa (2012) showed that seedlings of *Clethra barbinervis* preferentially regenerate on brown-rotted logs than on logs with other decay types. Although the reason for this preference pertaining to a specific decay type remains unclear, it is expected that wood chemical properties such as pH may have some effect because a significant relationships between wood pH and seedling density were observed (Fukasawa 2012). Furthermore, growing evidence shows that wood decay type determines wood nutrient contents (Takahashi et al. 2000; Fukasawa 2015a) as well as microbial inhabitants such as N-fixing bacteria (Aho 1974; Jurgensen et al. 1989), mycorrhizal fungi (Tedersoo et al. 2008), and myxomycetes (Fukasawa et al. 2015) of CWD. Therefore, differences in decay type may influence tree seedlings not only directly by their physicochemical properties but also indirectly by affecting symbiotic, antagonistic, and decomposer communities (Bardgett and Wardle 2010).

Forest dieback events often create huge volumes of CWD (Heilmann-Clausen 2013). In Japan, Japanese red pine (*Pinus densiflora* Sieb. et Zucc.) is a dominant canopy tree species of temperate secondary forests, and pine wilt disease (PWD) has killed many adult pine trees over recent decades (Takemoto and Futai, 2008), generating substantial pine CWD in forests (Kato and Hayashi 2006). I recently found that decayed pine logs in the post-PWD forests present suitable regeneration sites for several tree species and that the decay type of the logs affects the seedling community (Fukasawa 2012). Because the decay type of pine logs could highly vary according to environmental variables such as climate (Fukasawa 2015b), seedling preferences pertaining to a specific decay type may be critical for their regeneration after PWD. Thus, the aim of the present study is to compare seedling growth of tree species regenerating in post-PWD forests on different substrates such as soil and decayed wood of distinct decay types. To separate the effects of chemical and biological factors on seedling performance, sterilized substrates were used as a counterpart of non-sterile substrates for each substrate type. Seedlings of 4 tree species were used: 1) *P. densiflora*, 2) *Cryptomeria japonica* (Thunb. Ex L.f) D. Don, 3) *Clethra barbinervis* Sieb. et Zucc., 4) *Eurya japonica* Thunb. The regeneration of those 4 species has frequently been recorded in post-PWD forests (Higo et al. 1995; Fujihara et al. 2002; Kato and Hayashi 2006, 2007) and also recorded as seedlings on decayed logs of *P. densiflora* (Fukasawa 2012, 2015a; Fukasawa and Komagata unpublished). I hypothesized that 1) species with high mycorrhizal dependence (including *P. densiflora*; Sim and Eom 2006) are strongly affected by a sterile treatment rather than substrate difference, 2) species with high nutrient demands (including *C. japonica*; Nakaji et al. 2001) are strongly associated with substrate differences rather than sterile treatment, and 3) the growth of *C. barbinervis* seedlings may be better on brown-rotted wood than white-rotted wood because the seedlings of this species regenerate preferentially on brown-rotted logs (Fukasawa 2012).

Materials and methods

Tree seedlings

Properties of the 4 tree species used for the current growth experiment are given in Table 1. The used species differed with respect to their family, growth form, seed weight, nutrient demand, regeneration site, nutrient requirement, and mycorrhizal type and dependency. Seeds of *P. densiflora* and *C. japonica* were obtained from the Forest Breeding Center of Forestry and Forest Products Research Institute (FFPRI) (Ibaraki, Japan). Seeds of *C. barbinervis* and *E. japonica* were obtained from adult trees at Higashiyamato Park, Tokyo (35°45′N, 139°26′E; 114–122 m a.s.l.) and at the Yamashiro Experimental Forest of FFPRI, Kyoto (34°47′N, 135°50′E; 255 m a.s.l.), respectively. Seeds were sanded with wet paper and stored at 4°C for more than 4 months to break dormancy. Subsequently, seed surfaces were sterilized

Table 1. Taxonomy and life-history traits of the 4 tree species used in the present study

Seedling	Family	Growth form	Seed fresh weight (mg)[1]	Regeneration site[2]	Nutrient requirement[3]	Mycorrhizal type[4]	Mycorrhizal dependency[5]
Pinus densiflora	Pinaceae	canopy	10.0	Decayed wood, exposed mineral soil	Low	ECM	High
Cryptomeria japonica	Cupressaceae	canopy	4.6	Decayed wood	High	AM	unknown
Clethra barbinervis	Clethraceae	shrub	0.1	Decayed wood, exposed mineral soil	unknown	AM	unknown
Eurya japonica	Pentaphylacaceae	shrub	1.0	Exposed mineral soil	unknown	AM	unknown

[1] Tateishi et al. 2001 (*P. densiflora*); Matsuda et al. 2015 (*C. japonica*); Kobayashi and Kamitani 2000 (*C. barbinervis*) ; Tsujino and Yumoto 2004 (*E. japonica*).
[2] Iwasaki et al. 1997, Obase et al. 2012 and Fukasawa 2015 (*P. densiflora*) ; Ota et al. 2015 (*C. japonica*); Kobayashi and Kamitani 2000 and Fukasawa 2012 (*C. barbinervis*); Manabe and Yamamoto 1997 (*E. japonica*).
[3] Nakaji et al. 2001 (*P. densiflora* and *C. japonica*).
[4] Yamato and Iwase 2005.
[5] Sim and Eom 2006 and Dalong et al. 2011 (*P. densiflora*).

with 30% hydrogen peroxide, and the seeds were germinated on moist cotton under sterilized conditions at 20°C (12h light, 12h dark).

Experimental design

Three substrates were prepared: 1) white-rotted wood, 2) brown-rotted wood, and 3) soil. All substrates were obtained from Higashiyamato Park. The vegetation of Higashiyamato Park is pine- and oak-dominated secondary forest and has been described by Fukasawa (2012). White- and brown-rotted woods were collected from the decayed logs of *P. densiflora*. Each substrate was collected from 3 locations in the park and mixed to avoid the effects of local differences in microbe flora specific to certain vegetation. Collected substrates were milled to pass a 6 mm screen using a Retsch® SM 300 cutting mill (Verder Scientific Co. Ltd., Germany). Half of each substrate was autoclaved at 121°C for 1 h to prepare sterile substrates. In total, 6 experiments (3 substrates by 2 sterile levels) were set up. In the substrates of the 6 experiments, the pH was measured using a pH meter (Horiba, Kyoto, Japan), whereas the concentrations of cations (Na^+, NH_4^+, K^+, Mg^{2+}, and Ca^{2+}) and anions (F^-, Cl^-, NO_2^-, Br^-, NO_3^-, SO_4^{2-}, and PO_4^{3-}) were measured using an ion chromatography system (ICS-1000/2000; Dionex, CA, USA). Details of the measurements of pH and ions have been described by Fukasawa (2015a). The ion concentrations were expressed as values per 1 g of dried substrate.

A transparent polypropylene case (7.2 × 7.2 × 10 cm) (Incu Tissue; SPL Life Sciences, Korea) was first filled with approximately 173 ml vermiculite (water content, 26%), following which an approximately 43 ml substrate was filled on the upper layer.

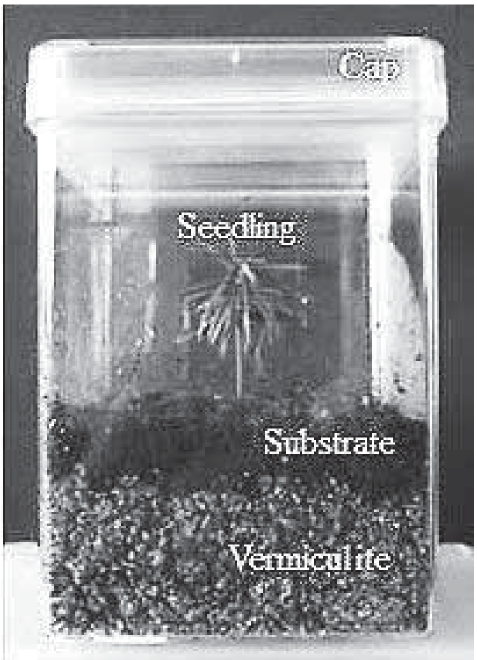

Fig. 1. Experimental microcosm system

A germinated seed was aseptically placed on the surface of the substrate in the case. All cases were capped with a transparent polypropylene cap and sealed with Parafilm® to prevent water evaporation (Fig. 1). Cases were incubated at 25°C under a 10 h dark and 14 h light cycle a day using a 11,000 lux fluorescent lamp for 4 months. Five replicated cases were prepared for each experiment and for each species. In total, 120 cases were incubated.

Measurements for retrieved seedlings

The retrieved seedlings were divided into aboveground shoots and underground roots. The heights of the shoots were measured and leaf numbers were counted. The shoots and roots were oven dried to a constant weight at 70°C and weighed. The shoot/root ratio (S/R) was calculated using the following equation:

S/R = shoot dry weight/root dry weight

Data analyses

A two-way analysis of variance (ANOVA) was applied to test the effects of substrate, autoclaving, and their interactions on pH and ion concentrations of substrates and seedling growth (shoot length, leaf number, shoot weight, root weight, and S/R). A Tukey's honest significant difference (HSD) post hoc test was performed to compare the values among the 6 experiments. A generalized liner model (GLM) was used to identify chemical properties associated with seedling growth. According to the correlations among the chemical factors (Table 3), pH, NH_4^+, K^+, and PO_4^{3-} were selected as variables. NH_4^+ has significant correlations with Mg^{2+}, Ca^{2+}, F^-, and Cl^- (Table 3); thus, it can act as an indicator of these elements. K^+ and PO_4^{3-} are the nutrients primarily important for plants in addition to nitrogen and have relatively less correspondence with other nutrients (Table 3). Furthermore, an alternative GLM with NO_3^-, K^+, and PO_4^{3-} as fixed factors was tested. In that case, data of autoclaved soil were not used because the NO_3^- concentration of autoclaved soil was beyond the measurable range of ion chromatography, and accordingly, pH was removed from the factors because of the limitation of factor numbers acceptable in the GLM model. All statistical tests were conducted using R 3.1.2 (R development core team 2014).

Results

Chemical properties of the substrates

pH and ion concentrations of the substrates were different among the 6 experiments (Figs. 2, 3). Decayed wood, particularly brown-rotted wood, had a significantly lower pH than soil. Autoclaving significantly reduced pH in all substrates. Nutrient ion concentrations were affected by substrate and autoclaving, except for Na^+, where standard errors of the data were large and significant effects were not detected (Table 2), although a trend of low Na^+ concentrations in brown-rotted wood compared with that in white-rotted wood and soil was evident (Fig. 3). Concentrations of NH_4^+ were significantly higher in autoclaved white-rotted wood and soil than brown-rotted wood and non-autoclaved white-rotted wood. K^+ was significantly and substantially higher in white-rotted wood than brown-rotted wood and soil. Mg^{2+} and Ca^{2+} were also significantly higher in white-rotted wood and soil than brown-rotted wood. F^- showed the same trend in autoclaved substrates but not in non-autoclaved substrates. A weak effect of substrates on Cl^- concentrations was detected by two-way ANOVA, whereas significant differences were not detected by Tukey's HSD post hoc test (Table 2). NO_3^- concentrations were extremely higher in non-autoclaved soil than other substrates, whereas the data for autoclaved soil were not obtained because the NO_3^- concentrations were below the limit of detection of our ion chromatography system. SO_4^{2-} concentrations were significantly higher in soil than wood. PO_4^{3-} concentrations were significantly higher in autoclaved woods than non-autoclaved woods and soil. To summarize, brown-rotted wood contained a low level of nutrients, and autoclaving increased nutrient concentrations.

Table 3 shows Pearson's correlation coefficients between the chemical properties of the substrates. Among the nutrient ions, NH_4^+ and F^- concentrations had significant correlations with 6 of the chemical properties measured. K^+, Mg^{2+}, Ca^{2+}, and Cl^- concentrations had significant correlations with 5 of the other properties. pH showed 4 correlations, SO_4^{2-} and PO_4^{3-} concentrations showed 3 correlations, and NO_3^- concentrations showed 2 correlations with other properties. Na^+ concentrations showed no correlations with other properties.

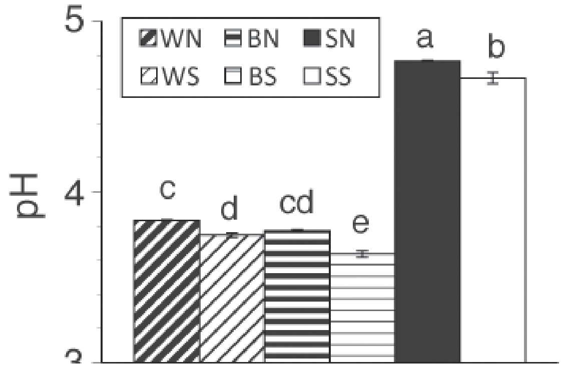

Fig. 2. pH of the substrates in the 6 experiments. WN, non-sterile white-rotted wood; WS, sterile white-rotted wood; BN, non-sterile brown-rotted wood; BS, sterile brown-rotted wood; SN, non-sterile soil; and SS, sterile soil.

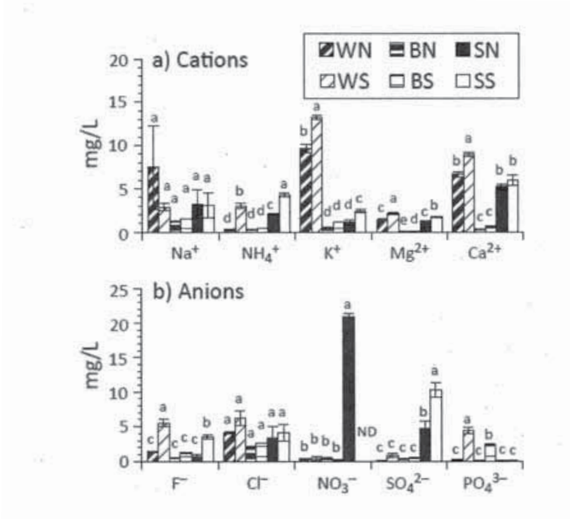

Fig. 3. Concentrations of the cations and anions in the substrates used in the 6 experiments. Abbreviations of the experiments are shown in Fig. 2.

Table 2. Results of two-way ANOVA evaluating effects of substrate, autoclaving, and their interaction on pH and nutrient ion concentrations in white- and brown-rotted wood and soil used as the substrates for seedling growth.

	Two-way ANOVA (*F* value)		
	Substrate	Autoclaving	Interaction
pH	2222.83 ***	61.50 ***	1.18
Cations			
Na^+	1.50	0.68	0.76
NH_4^+	130.50 ***	144.00 ***	31.70 ***
K^+	985.22 ***	71.02 ***	17.12 ***
Mg^{2+}	473.29 ***	78.26 ***	14.76 ***
Ca^{2+}	267.44 ***	17.75 **	5.10 *
Anions			
F^-	46.41 ***	137.87 ***	21.49 ***
Cl^-	4.72 *	2.32	0.39
NO_3^-	3545.68 ***	0.11	0.39
SO_4^{2-}	97.95 ***	19.46 ***	12.50 **
PO_4^{3-}	53.17 ***	171.23 ***	45.43 ***

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Table 3. Pearson's correlation coefficients between chemical properties of the substrates.

Variable	pH	Na^+	NH_4^+	K^+	Mg^{2+}	Ca^{2+}	F^-	Cl^-	NO_3^-	SO_4^{2-}	PO_4^{3-}
pH	1	0.02	0.62 **	-0.37	0.37	0.27	-0.03	0.004	0.98 ***	0.84 ***	-0.50 *
Na^+		1	-0.05	0.27	0.25	0.30	0.04	0.35	0.001	0.04	-0.15
NH_4^+			1	0.16	0.71 ***	0.60 **	0.74 ***	0.51 *	0.38	0.80 ***	0.17
K^+				1	0.71 **	0.79 ***	0.68 **	0.66 **	-0.38	-0.31	0.59 *
Mg^{2+}					1	0.98 ***	0.75 ***	0.66 **	0.17	0.40	0.28
Ca^{2+}						1	0.72 ***	0.72 ***	0.14	0.27	0.32
F^-							1	0.73 ***	-0.29	0.25	0.68 **
Cl^-								1	-0.06	0.20	0.42 +
NO_3^-									1	0.94 ***	0.33
SO_4^{2-}										1	-0.37
PO_4^{3-}											1

$^+P < 0.1$, $*P < 0.05$, $**\ P < 0.01$, $***\ P < 0.001$

Seedling properties

Fig. 4 shows the seedling properties measured. In *P. densiflora* seedlings, the shoot length was significantly longer in non-sterile soil than in sterile white- and brown-rotted woods, whereas root weight was significantly larger in non-sterile white-rotted wood than sterile brown-rotted wood and soil. S/R was significantly larger in soil than non-sterile white- and brown-rotted woods. Leaf number and shoot weight were not significantly different among the experiments. Using two-way ANOVA, a substrate effect was detected in the shoot length and S/R, whereas an effect of autoclaving was detected in shoot length, shoot weight, and root weight (Table 4). Interactions between substrate and autoclaving were not detected in any of the seedling properties measured.

In *C. japonica* seedlings, the aboveground growth (shoot length, shoot weight, and leaf number) was generally larger in soil than decayed wood, whereas root weight was not significantly different among the experiments. S/R was significantly larger in soil than wood in non-sterile experiments. Using two-way ANOVA, a significant substrate effect was detected in all properties tested, whereas an effect of autoclaving was not detected (Table 4). Significant interactions between substrate and autoclaving were not detected.

In *C. barbinervis* seedlings, shoot weight and leaf number were larger in non-sterile soil than non-sterile decayed woods. However, in sterile experiments, shoot weight and leaf number in brown-rotted wood were significantly larger than those in other woods and reached the same growth level as that achieved in non-sterile soil. Shoot length, root weight, and S/R

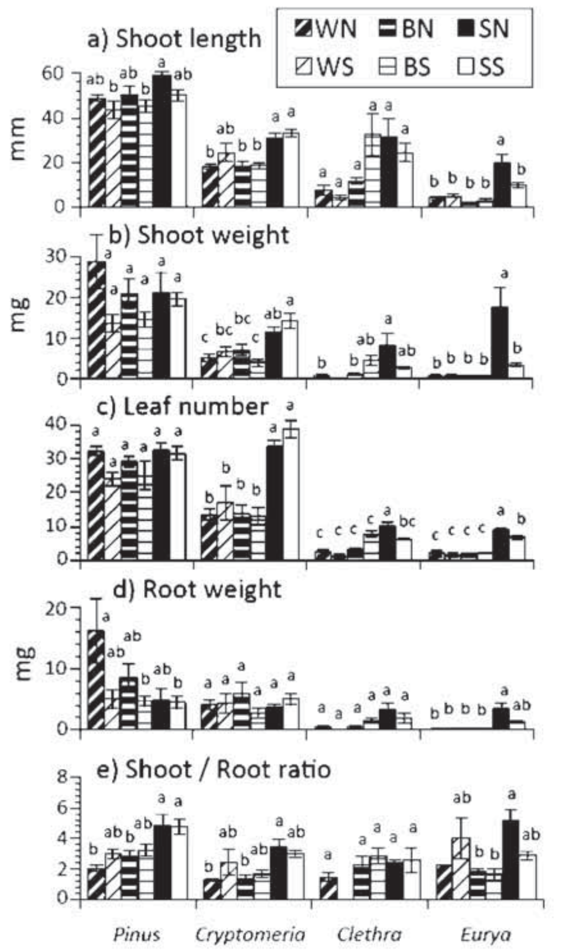

Fig. 4. Seedling properties of the 4 tree species in the 6 experiments. Abbreviations of the experiments are shown in Fig. 2.

Table 4. Results of two-way ANOVA evaluating effects of substrate, autoclaving, and their interaction on seedling growth on white- and brown-rotted wood and soil.

	Two way ANOVA (*F* value)		
	Substrate	Autoclaving	Interaction
Shoot length (mm)			
Pinus densiflora	4.0 *	7.3 *	0.4
Cryptomeria japonica	18.8 ***	2.3	0.9
Clethra barbinervis	3.9 *	0.3	2.1
Eurya japonica	25.3 ***	4.1 ⁺	5.9 *
Shoot weight (mg)			
Pinus densiflora	0.6	5.5 *	1.4
Cryptomeria japonica	22.0 ***	0.1	2.8 ⁺
Clethra barbinervis	4.4 *	0.6	4.2 *
Eurya japonica	14.8 ***	9.6 **	8.8 **
Leaf number			
Pinus densiflora	2.0	4.3 ⁺	0.9
Cryptomeria japonica	39.4 ***	1.2	0.6
Clethra barbinervis	26.5 ***	0.7	15.1 ***
Eurya japonica	107.3 ***	5.7 *	3.1 ⁺
Root weight (mg)			
Pinus densiflora	3.0 ⁺	5.3 *	2.0
Cryptomeria japonica	0.0	0.3	2.2
Clethra barbinervis	5.6 *	0.3	1.7
Eurya japonica	19.8 ***	7.0 *	6.4 **
Shoot / Root ratio			
Pinus densiflora	15.81 ***	1.47	0.69
Cryptomeria japonica	8.59 **	0.80	1.77
Clethra barbinervis	1.08	0.43	0.19
Eurya japonica	4.93 *	1.77	2.70 ⁺

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

were not significantly different among the experiments. Using two-way ANOVA, a substrate effect was detected in all properties, except S/R (Table 4). An effect of autoclaving was not detected, whereas significant interactions between substrate and autoclaving were detected in shoot weight and leaf number.

In *E. japonica* seedlings, above- and belowground growth was significantly larger in soil than other substrates particularly in the non-sterile experiment. S/R in non-sterile soil was larger than in brown-rotted wood. Using a two-way ANOVA, a significant substrate effect was detected in all properties (Table 4). Significant autoclaving effects were also detected on shoot weight, leaf number, and root weight. Significant interactions were detected on shoot length, shoot weight, and root weight.

Chemical factors associated with seedling properties

In *P. densiflora* seedlings, leaf number was negatively associated with PO_4^{3-}. Other properties of *P. densiflora* were not associated with chemical factors (Table 5). In *C. japonica* seedlings, shoot length was positively associated with pH and NH_4^+, whereas other seedling properties showed no relationships with chemical factors. Any measured properties of *C. barbinervis* seedlings were not associated with chemical factors. In *E. japonica* seedlings, leaf number was positively associated with pH and PO_4^{3-} and negatively associated with NH_4^+, whereas other seedling properties showed no relationships with chemical factors.

Discussion

The lower pH of rotten wood compared with soil observed in the present study, particularly the lower pH of brown-rotted wood, is in line with results of previous studies (Espejo and Agosin 1991; Takahashi et al. 2000; Fukasawa 2012, 2015a; Fukasawa et al. 2015). Organic acids released from saprobic fungi during the decay process reduce the pH of decaying woody substrates (Rayner and Boddy 1988), particularly in brown rot process where brown rot fungi use oxalic acids to generate hydroxyl radicals for non-enzymatic cell wall degradation (Espejo and Agosin 1991). Acidification after autoclaving may be partly caused by the enhanced ionization of nutrients, particularly F^-, NH_4^+, K^+, Mg^{2+}, Ca^{2+}, and PO_4^{3-} (Vacin and Went 1949; Matsui 1995). Sterilization of organic matter is known to cause leakage of nutrients immobilized in microbial inhabitants (Packer and Clay 2000; Troelstra et al. 2001; McCarthy-Neumann and Kobe 2010) and may result in the nutrient flush recorded in the present study. An additional cause of acidification by autoclaving may be attributed to the extraction of phenolic acids from decayed wood and plant residues in soil (Martens 2002). Hydrolysis of oligosaccharides such as sucrose could additionally contribute to a reduced pH (Druart and Wulf 1993); however, this may not have been the case in the current study because decayed wood contains very little soluble sugar components (Eaton and Hale 1993). Observation of high concentrations of nutrient ions in white-rotted wood in the current study were additionally consistent with previous reports (Takahashi et al. 2000; Fukasawa 2015a), although the reason for this trend remains unclear.

The present study clearly showed that substrate differences and autoclaving affect seedling growth, whereas the responses were species specific. *P. densiflora* seedlings were affected by both substrate and autoclaving. Autoclaving had much more effect than substrate on pine seedling growth; autoclaving reduced the above- and belowground seedling growth, probably attributable to the lack of symbiotic microbes in autoclaved substrates. It is well-known that ectomycorrhizal symbiosis is essential for the growth

Table 5. Generalized liner model results showing parameters estimated for chemical factors associated with seedling properties of each of the 4 tree species.

	pH	NH_4^+	K^+	PO_4^{3-}	NO_3^{-} †
Shoot length (mm)					
Pinus densiflora	1.46	-0.88	0.05	0.16	0.82 ⁺
Cryptomeria japonica	0.76 *	0.39 *	-0.06	0.19 ⁺	0.91
Clethra barbinervis	0.9	-0.47	-0.84	0.77	0.45
Eurya japonica	1.81 ⁺	-0.8	0.11	0.68	1.16 *
Shoot weight (mg)					
Pinus densiflora	0.18	-0.41	0.81 ⁺	-1.08 ⁺	-0.09
Cryptomeria japonica	0.30	0.67	-0.12	-0.34	0.68
Clethra barbinervis	1.72	-1.11	-0.48	1.06	0.86
Eurya japonica	1.98	-1.14	0.02	0.86	1.12 **
Leaf number					
Pinus densiflora	0.59 ⁺	-0.26	0.46 ⁺	-0.84 *	0.36
Cryptomeria japonica	0.65	0.41	-0.12	-0.04	0.81 ⁺
Clethra barbinervis	1.3	-0.76	-0.67	0.82	0.66
Eurya japonica	1.29 **	-0.31 *	-0.12 ⁺	0.29 *	0.97 *
Root weight (mg)					
Pinus densiflora	-0.27	-0.39	0.92 ⁺	-0.99	-0.49
Cryptomeria japonica	-1.43	1.27	0.03	-1.31	-0.41
Clethra barbinervis	1.48	-0.72	-0.43	0.69	0.87
Eurya japonica	1.83	-0.92	-0.05	0.74	1.11 **
Shoot / Root ratio					
Pinus densiflora	0.85 *	0.17 ⁺	-0.51 *	0.47 *	0.85 *
Cryptomeria japonica	1.29 ⁺	-0.12	-0.10	0.71	1.09 ⁺
Clethra barbinervis ††	–	0.40 *	-0.94 *	0.98 *	–
Eurya japonica	1.82	-0.82	0.39	0.95	1.20

† Calculated by alternative model.

†† Data of autoclaved soil were not applicable because very little root growth prevented S/R calculation and, accordingly, pH and NO_3^- were excluded from analysis because of the limitation of factor number available for the model.

of *P. densiflora* seedlings (Yamada and Katsuya 1996, 2001; Guerin-Laguette et al. 2004; Sim and Eom 2006; Ma et al. 2010, 2012; Dalong et al. 2011). Because well decayed wood contains a substantial number of ectomycorrhizal propagules (Kubartová et al. 2012; Rajala et al. 2011, 2012, 2015; Fukasawa and Matsuoka 2015), seedlings colonized on decayed wood can develop ectomycorrhizal root tips to the same extent as seedlings established on the ground (Christy et al. 1982; Vogt et al. 1995; Goodman and Trofymow 1998; Tedersoo et al. 2003, 2008, 2009; Baier et al. 2006; Buée et al. 2007; Elliott et al. 2007; Iwański and Rudawska 2007; Walker and Jones 2013; Fukasawa 2015a). Autoclaving destroys the entire community of ectomycorrhizal fungi in the substrates, which may cause a significant reduction in nutrient acquisition and growth of pine seedlings. Although substrate differences had little effect on the aboveground growth of pine seedlings, significant effects on root growth and S/R were evident. Smaller S/R values in *P. densiflora* seedlings grown on decayed wood than in soil were also reported within the field survey by Fukasawa (2015). Differences in plant root allocation between substrates are generally attributable to water and nutrient (particularly nitrogen) availability of the substrates (Lambers et al. 1998). Because in the present study, water content was regulated to be similar among experiments, the differences in plant root allocation were likely attributable to nutritional differences between substrates. Chiwa et al. (2012) showed that *P. densiflora* seedlings with good nitrogen uptake allocate more carbon to shoots than roots. In the present study, although NH_4^+ concentrations showed no significant associations with root growth and S/R in the GLM model, an alternative model with NO_3^- instead of NH_4^+, detected a significant positive effect of NO_3^- on S/R of *P. densiflora* seedlings. These results suggested that *P. densiflora* seedlings have high potential for morphological plasticity and can colonize both soil and decayed woods regardless of the decay type as long as the symbiotic fungal communities exist. Previous field observations additionally support the assertion of allocation plasticity and colonizability on a variety of microsites (Fukasawa 2015a; Han et al. 2015).

Growth, particularly aboveground growth, of *C. japonica* seedlings differed among substrates and was clearly explained by pH and NH_4^+, whereas growth was not affected by autoclaving. These results suggested that *C. japonica* seedlings prefer relatively high pH and nitrogen rich substrates rather than viability of soil symbiotic organisms. The sensitivity of *C. japonica* seedlings to NH_4^+ was also reported by Yamaguchi et al. (2014), where NH_4^+ substantially promoted the net photosynthetic rate in current-year needles of *C. japonica* seedlings, probably because of increased stomatal conductance and concentrations of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) and chlorophyll. The relationships between pH and seedling growth have additionally been extensively researched. Matsui (1995) reported that the acidic condition near pH 3.0 causes extensive damage to biological activity of *C. japonica* seedlings and found pH 3.2 to be the lower limit threshold of seedling tolerance in a water culture experiment, although the effects were reduced when humus-containing forest soil was used as a growth substrate (Hirano and Hijii 2000). Soil acidic conditions are known to reduce plant growth not only by the root-growth inhibition effect (Mallik 2003) but also by accelerating the release of toxic aluminium ions from soil particles (Kramer and Kozlowski 1979). Although the concentration of aluminium ions was not measured in the present study, Ostrofsky et al. (1997) reported that aluminium concentration in decayed wood is <8 μM even when the wood is decayed by certain brown rot fungi that accumulate relatively high concentrations of aluminium. This concentration (8 μM) is lower than the previously-tested minimum effect level on *C. japonica* growth (20 μM) (Hirano et al. 2007). *C. japonica* is an arbuscular mycorrhizal plant (Fujimaki et al. 2001). However, the importance of mycorrhizal colonization on the performance of their seedlings is rarely studied. In the present study, autoclaving of the substrates had no significant effects on *C. japonica* seedling growth, which was opposite to the response of *P. densiflora* seedlings. These results suggested that *C. japonica* seedlings are highly sensitive to the nutrient level of the substrates; thus, they are likely to preferentially colonize on soil rather than decayed wood.

The aboveground growth of *C. barbinervis* in brown-rotted wood was, though limited to the sterile experiment, recorded as the same level as that in soil, whereas less growth was recorded on white-rotted wood. This result is consistent with observations of previous field studies where the seedling density of *C. barbinervis* was high on brown-rotted wood

(Fukasawa 2012) as well as soil without a litter layer (Nakashizuka 1989; Kobayashi and Kamitani 2000). Because none of the chemical factors showed significant associations with seedling growth, the reason for the increased growth of *C. barbinervis* seedlings on brown-rotted wood than white-rotted wood remains unclear. *C. barbinervis* is an arbuscular mycorrhizal tree species, and it was suggested that mycorrhizal symbiosis is important for the growth of *C. barbinervis* seedlings on decayed logs (Fukasawa 2012). However, any evidence showing positive effects of fungal symbionts on *Clethra* seedling growth (i.e., greater growth in non-sterile experiments than sterile experiments) was not detected.

The above- and belowground growth of *E. japonica* was larger in non-sterile soil than wood and was positively associated with NO_3^- concentration. *E. japonica* is a small-seeded shrub species regenerating on exposed mineral soil on ridges or upper slopes in the forest understory (Manabe and Yamamoto 1997). Fukasawa (2012) reported that seedling density of *E. japonica* is larger on soil than decayed logs. The observation of good growth in soil in the present study is in line with these previous field observations and suggests that *E. japonica* seedlings have high nitrogen (notably nitrate) demands, although high nitrate demands are not consistent with their preference for ridges or upper slopes where nitrate-nitrogen is typically very low (Koyama et al. 2013).

In summary, the hypothesis that "species with high mycorrhizal dependence (*P. densiflora*) are strongly associated with a sterile treatment rather than substrate difference and species with high nutrient demands (*C. japonica*) are strongly associated with substrate difference rather than sterile treatment" was supported by the results of the present study. Similarly, the improved growth of *C. barbinervis* on brown-rotted wood than white-rotted wood supports my previous field observations (Fukasawa 2012) and confirms the preference for brown-rotted wood during seedling colonization. From the growth data of *P. densiflora*, *C. japonica,* and *E. japonica* on the 3 different substrates, it is expected that *P. densiflora* seedlings may be able to regenerate within the 3 substrates as long as mycorrhizal symbionts exist, whereas *C. japonica* and *E. japonica* seedlings may prefer soil rather than decayed wood because of the nutrient (especially nitrogen) conditions of soil. However, a recent report regarding regeneration microsites of *C. japonica* indicates that they prefer decayed stumps rather than soil (Ota et al. 2015). Such disagreement between laboratory and field observations provides motivation for further research and may result in the discovery of new and unexpected mechanisms of regeneration site preferences in forest tree species.

Acknowledgements

The author extends sincere thanks to the following persons in Field Science Center of Tohoku University for their technical support: Kanako Kimura for cation and anion measurement by ion chromatography; Ryo Uno for milling decayed wood materials. I also thank stuffs of Higashiyamato park, Forest Breeding Center, and Yamashiro experimental forest of FFPRI for providing tree seeds. This study was supported financially by Japan Society for the Promotion of Science (JSPS) KAKENHI Grant Number 23780156 to YF. The author would like to thank Enago (www.enago.jp) for the English language review.

References

Aho, PE., RJ. Seidler, HJ. Evans and PN. Raju (1974) Distribution, enumeration, and identification of nitrogen-fixing bacteria associated with decay in living white fir trees. Phytopathol, 64: 1413-1420.

Bače, R., M. Svoboda, V. Pouska, P. Janda and J. Červenka (2012) Natural regeneration in Central-European subalpine spruce forests: Which logs are suitable for seedling recruitment? Forest Ecol Manage, 266: 254-262.

Baier, R., R. Ettl, C. Hahn, and A. Gottlein (2006) Early development and nutrition of Norway spruce (*Picea abies* (L.) Karst.) seedling on different seedbeds in the Bavarian limestone Alps – a bioassay. Ann For Sci, 63: 339-348.

Bardgett, RD. and DA. Wardle (2010) Aboveground-belowground linkages: biotic interactions, ecosystem processes, and global change. Oxford University Press, Oxford.

Buée, M., PE. Courty, D. Mignot, and J.Garbaye (2007) Soil niche effect on species diversity and catabolic activities in an ectomycorrhizal fungal community. Soil Biol Biochem, 39: 1947-1955.

Cheng, D. (1989) Studies on *Racodium therryanum*-caused in-soil seed rot with special reference to its impact on natural regeneration of Yezo spruce.

Bulletin of the college experiment forests Hokkaido University, 46: 529-575. (in Japanese with English abstract)

Cheng, D. and T. Igarashi (1987) Fungi associated with natural regeneration of *Picea jezoensis* Carr. in seed stage: their distribution on forest floors and pathogenicity to the seeds. Bulletin of the college experiment forests Hokkaido University, 44: 175-188.

Chiwa, M., T. Matsuda, N. Nakatani, T. Kobayashi, A. Kume and H. Sakugawa (2012) Effects of canopy N uptake on folier CO_2 assimilation rates and biomass production and allocation in Japanese red pine seedlings. Can J For Res, 42: 1395-1403.

Dalong, M., W. Luhe, Y. Guoting, M. Liqiang, and L. Chun (2011) Growth response of *Pinus densiflora* seedlings inoculated with three indigenous ectomycorrhizal fungi in combination. Brazilian J Microbiol, 42: 1197-1203.

Doi, Y., AS. Mori and H. Takeda (2008) Conifer establishment and root architectureal responses to forest floor heterogeneity in an old-growth subalpine forest in central Japan. For Ecol Manage, 255: 1472-1478.

Christy, EJ., P. Sollins and JM. Trappe (1982) First-year survival of *Tsuga heterophylla* without mycorrhizae and subsequent ectomycorrhizal development on decaying logs and mineral soil. Can J Bot, 60: 1601-1605.

Druart, P. and OD. Wulf (1993) Activated charcoal catalyses sucrose hydrolysis during autoclaving. Plant Cell, Tissue and Organ Culture, 32: 97-99.

Eaton, RA. and MDC. Hale (1993) Wood: decay, pests and protection. Chapman & Hall

Elliott, JC., JE. Smith, K. Cromack Jr., H. Chen and D. McKay (2007) Chemistry and ectomycorrhizal communities of coarse wood in young and old-growth forests in the Cascade Range of Oregon. Can J For Res, 37: 2041-2051.

Espejo, E. and E. Agosin (1991) Production and degradation of oxalic acid by brown rot fungi. Appl Environ Microbiol, 57: 1980-1986.

Fujihara, M., Y. Hada and G. Toyohara (2002) Changes in the stand structure of a pine forest after rapid growth of *Quercus serrata* Thunb. For Ecol Manage, 170: 55-65.

Fujimaki, R., T. Tateishi, A. Kohzu, M. Saito and N. Tokuchi (2001) Characterization of arbuscular mycorrhizal colonization of 4 plant species in a Japanese red cedar plantation. Soil Microorganisms, 55: 121-128.

Fukasawa, Y. (2012) Effects of wood decomposer fungi on tree seedling establishment on coarse woody debris. For Ecol Manage, 266: 232-238.

Fukasawa, Y. (2015a) Basidiomycetous ectomycorrhizal fungal communities of curren-year *Pinus densiflora* seedlings that regenerated on decayed logs and on the forest floor soil. Journal of Integrated Field Science, 12: 19-30.

Fukasawa, Y. (2015b) The geographical gradient in pine log decomposition in Japan. For Ecol Manage, 349: 29-35.

Fukasawa, Y. and S. Matsuoka (2015) Communities of wood-inhabiting fungi in dead pine logs along a geographical gradient in Japan. Fung Ecol, 18: 75-82.

Fukasawa, Y., K. Takahashi, T. Arikawa, T. Hattori and N. Maekawa (2015) Fungal wood decomposer activities influence community structures of myxomycetes and bryophytes on coarse woody debris. Fung Ecol, 14: 44-52.

Guerin-Laguette, A., K. Shindo, N. Matsushita, K. Suzuki and F. Lapeyrie (2004) The mycorrhizal fungus *Tricholoma matsutake* stimulates *Pinus densiflora* seedling growth in vitro. Mycorrhiza, 14: 397-400.

Goodman, DM. and JA. Trofymow (1998) Distribution of ectomycorrhizas in microhabitats in mature and old-growth stands of Douglas-fir on southern Vancouver island. Soil Biol Biochem, 30: 2127-2138.

Han, S., SJ. Lee, TK. Yoon, SH. Han, J. Lee, S. Kim, J. Hwang, MS. Cho and Y. Son (2015) Species-specific growth and photosynthetic responses of first-year seedlings of four coniferous species to open-field experimental warming. Turkish J Agriculture and Forestry, 39: 342-349.

Harmon, ME. and JF. Franklin (1989) Tree seedling on logs in *Picea-Tsuga* forests of Oregon and Washington. Ecology, 70: 48-59.

Heilmann-Clausen, J. (2013) Dieback of European ash (*Fraxinus excelsior*) – Sheer misery or an opportunity for biodiversity? – Reply to Pautasso. Biol Conserv, 167: 450-451.

Higo, M., A. Shinohara and S. Kodama (1995) The regeneration behavior of major component species in the secondary forest dominated by Pinus densiflora and *Quercus serrata* in central Japan. For

Ecol Manage, 76: 1-10.

Hirano, Y. and N. Hijii (2000) Root system responses of Japanese red cedar saplings to acidic conditions. Environ Experim Bot, 44: 115-124.

Hirano, Y., T. Mizoguchi and I. Brunner (2007) Root parameters of forest trees as sensitive indicators of acidifying pollutants: a review of research of Japanese forest trees. J For Res, 12: 134-142.

Iwański, M. and M. Rudawska (2007) Ectomycorrhizal colonization of naturally regenerating *Pinus sylvestris* L. seedlings growing in different microhabitats in boreal forest. Mycorrhiza, 17: 461-467.

Iwasaki, Y., K. Sakamoto, K. Yoshikawa and K. Chiba (1997) Effects of light and forest floor conditions on the initial phase of regeneration of *Pinus densiflora* Sieb. et Zucc. in pine wilt disease forests. J Jpn For Soc, 79: 29-36. (in Japanese with English abstract)

Jurgensen, MF., MJ. Larsen, M. Wolosiewicz and AE. Harvey (1989) A comparison of dinitrogen fixation rates in wood litter decayed by white-rot and brown-rot fungi. Plant Soil, 115: 117-122.

Kato, J. and I. Hayashi (2006) Quantitative analysis of a stand of *Pinus densiflora* undergoing succession to *Quercus mongolica* ssp. *crispura*: I. A 31-year record of growth and population dynamics of the canopy trees. Ecol Res, 21: 503-509.

Kato, J. and I. Hayashi (2007) Quantitative analysis of a stand of *Pinus densiflora* undergoing succession to *Quercus mongolica* ssp. *crispura*: II. Growth and population dynamics of *Q. mongolica* ssp. *crispula* under the *P. densiflora* canopy. Ecol Res, 22: 527-533.

Kobayashi, M. and T. Kamitani (2000) Effects of surface disturbance and light level on seedling emergence in a Japanese secondary deciduous forest. J Veg Sci, 11: 93-100.

Kohyama, T. and PJ. Grubb (1994) Below- and above-ground allometries of shade-tolerant seedlings in a Japanese warm-temperate rain forest. Funct Ecol, 8: 229-236.

Koyama, L., M. Hirobe, K. Koba, and N. Tokuchi (2013) Nitrate-use traits of understory plants as potential regulators of vegetation distribution on a slope in a Japanese cedar plantation. Plant Soil, 362: 119-134.

Kramer, PJ. and TT. Kozlowski (1979) Physiology of woody plants. Academic Press, San Diego.

Kubartová, A., E. Ottosson, A. Dahlberg and J. Stenlid (2012) Patterns of fungal communities among and within decaying logs, revealed by 454 sequencing. Mol Ecol, 21: 4514-4532.

Lambers, H., FS. Chapin, and TL. Pons (1998) Plant physiological ecology. Springer.

Leck, MA., and HA. Outred (2008) Seedling natural history. In: Seedling ecology and evolution, Leck, MA., VT. Parker and RL. Simpson (eds), pp. 17-55, Cambridge University Press, Cambridge.

Ma, D., G. Yang, and L. Mu (2010) Morphological and molecular analyses of ectomycorrhizal diversity in *Pinus densiflora* seedlings. Symbiosis, 51: 233-238.

Ma, D., S. Zang, L. Wan and D. Zhang (2012) Ectomycorrhizal community structure in chronosequences of *Pinus densiflora* in eastern China. African J Microbiol Res, 6: 6204-6209.

Mallik, AU. (2003) Conifer regeneration problems in boreal and temperate forests with ericaceous understory: role of disturbance, seedbed limitation, and keystone species change. Crit Rev Plant Sci, 22: 341-366.

Manabe, T. and S. Yamamoto (1997) Spatial distribution of *Eurya japonica* in an old-growth evergreen broad-laeved forest, SW Japan. J Veg Sci, 8: 761-772.

Matsuda, O., M. Hara, H. Tobita, K. Yazaki, T. Nakagawa, K. Shimizu, A. Uemura, and H. Utsugi (2015) Determination of seed soundness in conifers *Cryptomeria japonica* and *Chamaecyparis obtusa* using narrow-multiband spectral imaging in the short-wavelength infrared range. PLOS ONE: DOI: 10.1371/journal.pone.0128358

Matsui, H. (1995) Effects of acidity of the water culture solution on uptake of nutrients by the root of Japanese cedar (*Cryptomeria japonica*) and Japanese cypress (*Chamaecyparis obtusa)*. Water, Air and Soil Pollution, 85: 1131-1136.

Martens, DA. (2002) Relationship between plant phenolic acids released during soil mineralization and aggregate stabilization. Soil Sci Soc Am J, 66: 1857-1867.

McCarthy-Neumann, S. and RK. Kobe (2010) Conspecific and heterospecific plant-soil feedbacks influence survivorship and growth of temperate tree seedlings. J Ecol, 98: 408-418.

Nakaji, T., M. Fukami, Y. Dokiya and T. Izuta (2001) Effects of high nitrogen load on growth, photosynthesis and nutrient status of *Cryptomeria japonica* and *Pinus densiflora* seedlings. Trees, 15: 453-461.

Nakashizuka, T. (1989) Role of uprooting in composition and dynamics of an old-growth forest in Japan. Ecology, 70: 1273-1278.

Nakashizuka, T. (2001) Species coexistence in temperate, mixed deciduous forests. Trends Ecol Evol, 16: 205-210.

Obase, K., JY. Cha, JK. Lee, SY. Lee and KW. Chun (2012) Ectomycorrhizal fungal community associated with naturally regenerating *Pinus densiflora* Sieb. et Zucc. seedlings on exposed granite slopes along woodland paths. J For Res, 17: 388-392.

O'Hanlon-Manners, DL. and PM. Kotanen (2004) Logs as refuges from fungal pathogens for seeds of eastern hemlock (*Tsuga canadensis*). Ecology, 85: 284-289.

Ostrofsky, A., J. Jellison, KT. Smith, and WC. Shortle (1997) Changes in cation concentrations in red spruce wood decayed by brown rot and white rot fungi. Can J For Res, 27: 567-571.

Ota, T., H. Sugita, T. Kanazashi and T. Masaki (2015) Seedling dynamics of *Cryptomeria japonica* in relation to difference of regeneration substrata. J Jpn For Soc, 97: 10-18. (in Japanese with English abstract)

Packer, A. and K. Clay (2000) Soil pathogens and spatial patterns of seedling mortality in a temperate tree. Nature, 404: 278-281.

Rajala, T., M. Peltoniemi, J. Hantula R. Mäkipää, and T. Pennanen (2011) RNA reveals a succession of active fungi during the decay of Norway spruce logs. Fung Ecol, 4: 437-448.

Rajala, T., M. Peltoniemi, T. Pennanen, and R. Mäkipää (2012) Fungal community dynamics in relation to substrate quality of decaying Norway spruce (*Picea abies* [L.] Karst.) logs in boreal forests. FEMS Microbiol Ecol, 81: 494-505.

Rajala, T., T. Tuomivirta, T. Pennanen and R. Mäkipää (2015) Habitat models of wood-inhabiting fungi along a decay gradient of Norway spruce logs. Fung Ecol, 18: 48-55.

Rayner, ADM. and L. Boddy (1988) Fungal decomposition of wood. Wiley, Chichester.

R development core team (2014) R: a language and environment for statistical computing R Foundation for Statistical Computing. Vienna, Austria.

Sim, MY. and AH. Eom (2006) Effects of ectomycorrhizal fungi on growth of seedlings of *Pinus densiflora*. Mycobiol, 34: 191-195.

Takahashi, I. (1991) Studies on the mycofloral succession on Yezo spruce (*Picea jezoensis* Carr.) in various stages of growth, with special reference to the role of fungi in the natural regeneration of the host. Bulletin of the University of Tokyo Forests, 86: 201-273. (in Japanese with English abstract)

Takahashi, M., Y. Sakai, R. Ootomo and M. Shiozaki (2000) Establishment of tree seedlings and water-soluble nutrients in coarse woody debris in an old-growth *Picea-Abies* forest in Hokkaido, northern Japan. Can J For Res, 30: 1148-1155.

Takemoto, S. and K. Futai (2008) Rapidity of disease development seems to result in high mortality: insight from an inoculation test using hybridized populations between a virulent and an avirulent isolates of *Bursaphelenchus xylophilus*. In: Pine wilt disease: a worldwide threat to forest ecosystems, Mota, MM. and P. Vieira (Eds), pp. 303-311, Springer, Berlin Heidelberg.

Tateishi, T., A. Kohzu, H. Yukutake and E. Wada (2001) Effects of seed size of Japanese red pine Pinus densiflora on colonization of pine seedlings by ectomycorrhizal basidiomycete Suillus granulatus and initial growth of seedlings. Soil Microorganisms, 55: 45-53. (in Japanese with English abstract)

Tedersoo, L., U. Kõljalg, N. Hallenberg and KH. Larsson (2003) Fine scale distribution of ectomycorrhizal fungi and roots across substrate layers including coarse woody debris in a mixed forest. New Phytol, 159: 153-165.

Tedersoo, L., T. Suvi, T. Jairus, and U. Kõljalg (2008) Forest microsite effects on community composition of ectomycorrhizal fungi on seedlings of *Picea abies* and *Betula pendula*. Environm Microbiol, 10: 1189-1201.

Tedersoo, L., G. Gates, CW. Dunk, T. Lebel, TW. May, U. Kõljalg and T. Jairus (2009) Establishment of ectomycorrhizal fungal community on isolated *Nothofagus cunninghamii* seedlings regenerating on dead wood in Australian wet temperate forests: does fruit-body type matter? Mycorrhiza, 19: 403-416.

Troelstra, SR., R. Wagenaar, W. Smart and BAM. Peters (2001) Interpretation of bioassays in the study of interactions between soil organisms and plants: involvement of nutrient factors. New Phytol, 150: 697-706.

Tsujino, R. and T. Yumoto (2004) Effects of sika deer on tree seedlings in a warm temperate forest on Yakushima Island, Japan. Ecol Res, 19: 291-300.

Vacin, EF. and FW. Went (1949) Some pH changes in nutrient solutions. Bot Gaz, 110: 605-613.

Vogt, KA., DJ. Vogt, H. Asbjornsen and RA. Dahlgren (1995) Roots, nutrients and their relationship to spatial patterns. Plant Soil, 168-169:113-123.

Walker, JKM. and MD. Jones (2013) Little evidence for niche partitioning among ectomycorrhizal fungi on spruce seedling planted in decayed wood versus mineral soil microsites. Oecologia, 173: 1499-1511.

Yamada, A. and K. Katsuya (1996) Morphological classification of ectomycorrhizas of *Pinus densiflora*. Mycoscience, 37: 145-155.

Yamada, A. and K. Katsuya (2001) The disparity between the number of ectomycorrhizal fungi and those producing fruit bodies in a *Pinus densiflora* stand. Mycol Res, 105: 957-965.

Yamaguchi, M., Y. Otani, P. Li, H. Nagao, IW. Lenggoro, A. Ishida, K. Yazaki, K. Noguchi, S. Nakaba, K.Yamane, K. Kuroda, Y. Sano, R. Funada and T. Izuta (2014) Effects of long-term exposure to ammonium sulfate particles on growth and gas exchange rates of *Fagus crenata*, *Castanopsis sieboldii*, *Larix kaempferi* and *Cryptomeria japonica* seedlings. Atmospheric Environment, 97: 493-500.

Yamato, M. and K. Iwase (2005) Community analysis of arbuscular mycorrhizal fungi in a warm-temperate deciduous broad-leaved forest and introduction of the fungal community into the seedlings of indigenous woody plants. Mycoscience, 46: 334-342.

Relationship of Serum Oxytocin Concentration to Positive Social Behaviors in Cattle

Siyu CHEN[1], Sanggun ROH[2] , Shusuke SATO[3]* and Shin-ichiro OGURA[1]

[1]Laboratory of Land Ecology, Graduate School of Agricultural Science, Tohoku University, Osaki, Miyagi, 989-6711, Japan
[2]Laboratory of Animal Physiology, Graduate School of Agricultural Science, Tohoku University, Miyagi, 981-8555, Japan
[3]Animal science, Faculty of Life & Environmental Science, Teikyo University of Science, Tokyo, 120-0045, Japan

Corresponding Author: Shin-ichiro OGURA, shin-ichiro.ogura.el@tohoku.ac.jp

Keywords: behavior, calves, cows, serum oxytocin

Received 27 January 2017; accepted 7 February 2017

Abstract

In order to study the relationship of serum oxytocin concentration to behavior in cows, two types of experiments were used: 1) calves were introduced to new rearing conditions; and 2) an event was created (new access to silage) in a stable herd where cows were familiar with other individuals. For the first experiment, fourteen Japanese Black calves (equal in gender) were used: six calves were named “introduced calves” and the other eight were named “group calves” and were split into two four-calf groups. Introduced calves were individually moved into the four-calf groups, and social behaviors (explorative behavior and affiliative behavior) were observed for the introduced calves from 1000 h to 2000 h (first day of introduction) and 0830 h (start of feeding) to 1030 h by continuous sampling. Blood samples were also collected from the introduced calves before and after the observational period. For the second experi ment, a stable herd of 16 Holstein milking cows was used. Social behaviors were observed for 3 h/day immediately after having access to grass silage for a total of six times. Blood samples were collected three times after finishing the behavioral observation and were used for the analysis of serum oxytocin concentrations. Results showed that serum oxytocin concentration positively correlated with explorative behavior in the calves (n = 6, r = 0.81, P = 0.05), and that serum oxytocin concentration significantly correlated with social affiliative behavior in the herd of Holstein cows (n = 16, r = 0.58, P = 0.02). These results suggest that the higher the serum oxytocin concentration is, the more explorative and social affiliative behavior are observed in cattle. Therefore, increases in serum oxytocin levels may improve the welfare of livestock animals as it enhances positive social behavior.

1. Introduction

Recently, oxytocin has been shown to be of increasing importance in terms of animal welfare (Broom and Fraser, 2007), since it plays an important role in positive social behaviors such as maternal behavior (Da et al., 1996; Williams et al., 2001), affiliative behavior (Winslow et al., 1993; Babygirija et al., 2012), and social grooming behavior in rats (Amico et al., 2004) and monkeys (Winslow and Insel, 1991). It is also known that oxytocin mediates pleasure events and positive interactions (Uvnäs-Moberg, 1998). For example, massage-like stroking of the abdomen lowers blood pressure, and this effect was diminished by intravenous injection of oxytocin antagonist in rats (Kurosawa et al., 1996). The supportive interaction “warm touch” between married couples increased their salivary oxytocin concentrations (Holt-Lunstad et al., 2008). These findings suggest that oxytocin may improve animal welfare through enhancing social grooming behavior, social bond generation, and stabilization of social bonds in herds.

On the other hand, farm animals are often forced to experience changes in rearing conditions (e.g., weaning, grouping of herds) during their lifetimes. These environmental changes affect an animal's behavioral and physiological indicators. Kondo et al. (1984) observed behavioral changes in Holstein calves (i.e., changes in activity, spatial patterns of laying, and aggressive behavior) after grouping. Mench et al. (1990) showed significant increases in serum cortisol concentrations of beef cows after regrouping, suggesting that regrouping causes behavioral and physiological changes in adaptation in calves. Social grooming, a positive social behavior, is thought to have functional significance in the formation and maintenance of social bonds and the stabilization of social relationships in cows (Sato et al., 1991).

However, there is scarce information on oxytocin in cattle. We recently found that serum oxytocin concentrations in calves were different among individuals under the same rearing system (Chen et al., 2014). Hence, it is hypothesized that there is a positive relationship between serum oxytocin concentrations in individuals and positive social behaviors in cattle.

In the present study, we investigated the relationship of serum oxytocin concentration to positive social behaviors in cattle under different rearing conditions. First, we studied the relationship of serum oxytocin concentration to positive social behaviors of calves when they were introduced into a novel herd and faced new mates. Second, we studied the relationship of serum oxytocin concentration to positive social behaviors of cows in a stable herd where they were familiar with other individuals.

2. Materials and methods

This study was carried out at the Field Science Center, Graduate School of Agricultural Science, at Tohoku University, Japan. Fourteen Japanese Black calves (equal in gender, 3.0 ± 1.1 months old) were used in the first experiment, and 16 Holstein milking cows (47.1 ± 24.1 months old) were used in the second experiment. Both experiments were approved by the president of Tohoku University, Sendai, Japan (Approval number: 2014 agriculture animal-61 and 2014 agriculture animal-65).

2.1.1 Animal management

Six calves (equal in gender) were used as “introduced animals.” These animals would be moved into a stable herd of other calves. The six calves were separated from their dams the day after delivery. Then, they were reared individually under a bucket suckling system in a single pen (1 m × 2 m). The calves were spatially, but not visually or acoustically isolated from each other. They were fed commercial artificial milk powder (All-in-One, Tokyo, Japan) dissolved in warm water (300 g/3 L) twice daily at 0840 h and 1540 h through a suckling bucket with a nipple. Then, after they were seven days old, calves were fed hay and starter (JA group, Miyagi, Japan). Water was provided *ad libitum*.

The other 8 calves were raised with their dams after delivery and allowed to suckle freely in a nursing barn. Two weeks before the beginning of the experiment, two groups of four calves each (i.e., 8 calves in total) were moved to another barn and were reared in a group in a 4 m × 6 m pen. The barn was 500 m from the single pen with the introduced calves; therefore, the introduced calves were completely segregated from the group calves. The amount of the starter was determined so that the calves gained greater than 0.6 kg/d (NARO, 2008) of body weight. Water and salt was provided *ad libitum*.

2.1.2 Behavioral observation and blood collection of the introduced calf

A 75-day-old calf was introduced into each four-calf group by two animal managers familiar with the calves. Calves in the two four-calf groups were 125.1 ± 9.0 days and 127 ± 14 days old (mean ± SD). Three video cameras (KJH-F 690, Samsung Co. Ltd., Seoul, Republic of Korea) connected to one recorder (H210, Panasonic Co. ltd., Tokyo, Japan) were installed one week before the test. It was assumed that behaviors would change after introduction and feeding. Thus, behaviors of the introduced calf were observed (i.e., 1000 h [(the beginning of introduced time)] to 2000 h, and 0830 h [feeding time] to 1030 h) by a continuous sampling method. During these periods, the number of self-grooming behaviors, social affiliative behaviors, received social affiliative behaviors, jumps, agonistic behaviors, received agonistic behaviors, and explorative behaviors were recorded. The above behavior types are described in Table 1.

Blood samples were collected from the introduced calf five days before test commencement and after observations were finished (1100 h). A 15 mL blood sample was collected from the calf in three 5-mL tubes, each containing 250 KIU aprotinin. The tubes were kept on ice until centrifugation (1,600 × *g* for 20

Table 1. Definition of behaviors analyzed in the both tests of calves and cows.

Behavioral type	Definition
Frequency of self-grooming behavior	Licks body or scratches head with a foot by one-self One sequence of several licks or scratches was counted as one
Frequency of social affiliative behavior	Licks or sniffs another's body One sequence of several licks or sniffs was counted as one
Frequency of received social affiliative behavior	Receives Lick or sniff from another One sequence of several licks or sniffs was counted as one
Frequency of jumping	Jumps with 4 legs off the floor
Frequency of agonistic behavior	Threatens another using head
Frequency of received agonistic behavior	Receives head threat from another
Frequency of explorative behavior	Sniffs or touches wall or floor with nose or tongue
Frequency of social affiliative behavior	Licks or sniffs another's body One sequence of several licks or sniffs was counted as one
Duration of self-grooming behavior	Duration of body licking or head scratching with a foot by one-self
Duration of social affiliative behavior	Duration of licking or sniffing another's body

min). After centrifugation, serum samples were stored at -80°C until oxytocin analysis was performed.

2.2.1 Management of Holstein milking herd

The Holstein milking herd consisted of 16 milking cows and was milked twice a day at 0840 h and 1600 h. The barn consisted of an indoor free lying area and an outdoor sport area. The indoor lying area was covered with rice hulls and was parted into independent 1.4 m × 1.9 m beds. The outdoor sport area had a concrete floor, in the middle of which there was a feeder (3 m × 4 m). The cows had free access to grass silage in the feeder. Concentrates were also fed to cows, at about 1/3 of the milk yield, as specified by NARO (2006). Water and salt were always available to the cows.

2.2.2 Behavioral observation and blood collection of cows

Grass silage was provided in the feeder at 1100 h twice a week (Monday and Thursday). All the cows were released into the outdoor sport area immediately after the new grass silage was provided. They were kept in the outdoor area until the end of behavioral observations. Behavioral observation was carried out for 3 h/day, for a total of 18 h over six days in three successive weeks (twice a week). In order to record cow behavior, four cameras (Sony DCR-SR 220, Sony Co. ltd., Tokyo, Japan) were installed on the wall one week before test commencement. The frequency and duration of self-grooming behavior, social affiliative behavior, and the frequency of agonistic behavior and received agonistic behavior were recorded. The above behavior types are described in Table 1.

Blood samples were collected once a week (a total of 3 times) from the jugular vein of the cows after the end of the behavioral observations at 1430 h. Serum samples were obtained according to the previously described method.

2.3. Measurement of serum oxytocin concentration

Serum samples were used for the analysis of oxytocin concentrations. Oxytocin concentrations were measured using an enzyme-linked immunosorbent assay (ELISA). Rabbit anti-oxytocin purified IgG (G-051-01, Phoenix Pharmaceuticals, Inc., Burlingame, CA, USA) was diluted to 57 ng/mL in coating buffer (0.015 M Na_2CO_3 and 0.034 M $NaHCO_3$: pH 9.6). Then, 100 µL of rabbit anti-oxytocin purified IgG was placed into a 96-well plate (445101, Thermo Scientific Nunc™, Denmark). After 24 h incubation at 4°C, the 96-well plate was washed with washing buffer (0.05% Tween 80) twice, and 250 µL blocking buffer (0.04 M Na_2HPO_4, 0.145 M NaCl, 0.1% BSA:

pH 7.2) was added to each well. After 2 h at room temperature, the plate was washed with washing buffer twice. Oxytocin standards and serum samples (100 µL) were added to the wells. After 24 h of incubation at 4°C, the plate was washed with washing buffer twice. Biotin-labeled oxytocin (AnaSpec Inc., San Jose, CA, USA) was diluted to 1 µg/mL in assay buffer (0.042 M Na_2HPO_4, 0.008 M KH_2PO_4, 0.02 M NaCl, 0.0048 M EDTA 1.79 g, 0.05% BSA: pH 7.5). Then, 100 µL of diluted biotin-labeled oxytocin was added to each well. After 24 h incubation at 4°C, the plate was washed twice with washing buffer. Streptavidin peroxidase (100 ng/well, SAP) (S5512, Sigma-Aldrich) diluted in assay buffer was added to each well, and the plate was stored at 4°C for 1 h. After that, the plate was washed with washing buffer twice, 100 µL TMB Soluble Reagent (High Sensitivity 23141, ScyTek Laboratories, Inc., Logan, UT, USA) was added to each well, and the plate was stored at 37°C for 30 min. The reaction was stopped by adding 25 µL of 2N HCl to each well. The absorbency at 450 nm was measured directly through the bottom of the plates with an ELISA Reader (International Reagents Corporation, Kobe, Japan). All other reagents were purchased from Wako Chemical Ltd. (Osaka, Japan).

2.4. Statistical analysis

In this study, the correlation coefficient was calculated to examine the relationship of serum oxytocin concentration to all the behaviors recorded. Probability values ≤ 0.05 were regarded as statistically significant and values ≤ 0.1 were considered to show a significant tendency. All the data were analyzed using SYSTAT 13 statistical software (SYSTAT Software, Inc., San Jose, CA, USA).

3. Results

3.1. Relationship between serum oxytocin concentration and behaviors in the introduced calves

Serum oxytocin concentrations in the introduced calves were 4.5 ± 1.3, 5.6 ± 0.5, 13.3 ± 5.2, 9.4 ± 3.0, 6.7 ± 1.8 and 7.9 ± 2.2 pg/ml (mean ± SD). Since serum oxytocin concentrations were not different between before and after the experimental period ($P > 0.05$), the mean value of the two samples was used for correlation analysis.

Serum oxytocin concentration positively correlated with explorative behavior in calves, but did not significantly correlate with other behaviors (r = 0.81, $P = 0.05$; n = 6). (Table 2)

3.2. Relationship between serum oxytocin concentration and behaviors in milking cows

Since serum oxytocin concentration was not different among the three sampling occasions ($P > 0.05$), the mean value of the three samples was regarded as a basal concentration and was used for correlation analysis.

Serum oxytocin concentration positively correlated with social affiliative behavior in milking cows (r = 0.58, $P = 0.02$; n = 16) (Table 3), but did not significantly correlate with other behaviors.

Table 2. Behaviors analyzed in introduced calf tests, and coefficient of behaviors with serum oxytocin concentration in calf response to new mates in new rearing conditions.

Behavioral type	No./cow/h (total 12 h) (mean ± SD)	Correlation to serum oxytocin concentration (n = 6)	
		r	*P*
Frequency of self-grooming behavior (No.)	4.9 ± 4.6	-0.03	0.91
Frequency of affiliative behavior (No.)	5.3 ± 5.2	0.51	0.30
Frequency of received affiliative behavior (No.)	3.2 ± 3.5	-0.01	0.99
Frequency of jumping (No.)	2.2 ± 2.1	0.25	0.64
Frequency of agonistic behavior (No.)	0.1 ± 0.1	-0.26	0.62
Frequency of received agonistic behavior (No.)	7.3 ± 7.5	0.35	0.46
Frequency of explorative behavior (No.)	6.7 ± 7.4	0.82	0.05

Table 3. Behaviors analyzed in the test of cows, and coefficient of behaviors with serum oxytocin concentration in a stable Holstein cows.

Behavioral type	No. or s/cow/h (Total 18 h) (mean ± SD)	Correlation to serum oxytocin concentration (n = 16)	
		r	*P*
Frequency of self-grooming behavior (No.)	0.7 ± 0.4	-0.17	0.53
Duration of self-grooming behavior (sec)	4.3 ± 2.4	-0.11	0.68
Frequency of social affiliative behavior (No.)	0.3 ± 0.2	0.58	0.02
Duration of social affiliative behavior (sec)	6.5 ± 0.2	0.09	0.73
Frequency of agonistic behavior (No.)	2.5 ± 1.8	0.19	0.47
Frequency of received agonistic behavior (No.)	2.5 ± 1.5	0.11	0.684

4. Discussion

4.1. Testing in calves

All domestic animals are strongly motivated to explore and investigate new environments. On some occasions, animals are placed in a novel situation that makes them fearful, and it has been stated that this fear may be connected to explorative motivation (Broom and Fraser, 2007). In the present study, the performed explorative behavior might be in response to the new environment and facing new mates. The positive correlation between serum oxytocin concentration and explorative behavior suggests that the higher the serum oxytocin concentration in a calf is, the less fearful the calf is when facing unfamiliar mates and a new environment. This result agrees with the finding that rats that have received oxytocin injections perform more normal behaviors after pro-conflict tests compared to rats in the control group, indicating that oxytocin increased the normal behavior pattern against stress (Svanidze et al., 2012). In addition, our previous study also supports this result that we found: the higher oxytocin concentration of calve is, the more social contacts with a decoy of Holstein calf in the open-field arena are observed (Chen et al., 2015).

4.2. Testing in cows

It has been reported that oxytocin induces affiliative behavior in rats (Babygirija et al., 2012) and voles (Rose and Young, 2009). This is in agreement with Dhakar et al. (2012), who showed that oxytocin receptor knockout rats performed more aggressive behaviors than the control rats. Dunbar (2008) reviewed that social grooming plays a particularly important role in social bonding and has a major impact on an individual's lifetime reproductive fitness. Furthermore, social grooming is thought to have functional significance for the formation and maintenance of social bonds and the stabilization of social relationships in cows (Sato et al., 1991). In the present study, we found that the higher the serum oxytocin concentration was, the more social affiliative behaviors were present in a stable herd of cows. However, it is hard to conclude that oxytocin plays an important role in the bonding and stability of a herd, because ages, body weights, and other factors may affect bonding and stability. Oxytocin did have a correlation with social affiliative behavior that promotes the establishment and stabilization of social bonds.

Da et al. (1996) mentioned central oxytocin induced maternal behavior in sheep and suggested that oxytocin has a positive feedback loop, in which oxytocin induced maternal behavior facilitates oxytocin release in both the brain and the blood. In addition, Neumann et al. (1994) reported that oxytocin acts in a positive feedback loop during suckling, suggesting that natural suckling may increase basal serum oxytocin concentrations in rats. This means that oxytocin concentration may induce positive social behavior, which in turn increases oxytocin concentration. Similarly, higher oxytocin concentrations may be beneficial to animals as they increase animals' stress tolerance, which may be explained by the mechanism in the review of Uvnäs-Moberg (1998).

In conclusion, it is suggested that a higher serum oxytocin concentration induces explorative and social affiliative behaviors in cattle as has been demonstrated for rats and humans. Since positive social behavior connects with improved welfare in cattle, studies on oxytocin in domestic animals may contribute greatly to improved animal management.

Acknowledgments

This study was supported by the Laboratory of Land Ecology, Graduate School of Agricultural Science, Tohoku University. We thank all the members in this laboratory for their support. We also thank Mr. Takashi Chiba, Mr. Shigefumi Tanaka, and Mr. Tetsuro Shishido of the Field Science Center, Graduate School of Agricultural Science, Tohoku University, for their valuable support.

References

Amico, J.A., R.R. Vollmer, J.R. Karam, P.R. Lee, X. Li, J.I. Koenig and M.M. McCarthy (2004) Centrally administered oxytocin elicits exaggerated grooming in oxytocin null mice. Pharmacology Biochemistry & Behavior, 78: 333–339.

Babygirija, R., D. Cerjak, S. Yoshimoto, I. Gribovskaja-Rupp, M. Bülbül, K. Ludwig and T. Takahashi (2012) Affiliative behavior attenuates stress responses of gi tract via up-regulating hypothalamic oxytocin expression. Autonomic Neuroscience: Basic and Clinical, 169: 28–33.

Broom, D.M. and A.F. Fraser (2007) Welfare Assessment in Direct Measures of Good Welfare. In Domestic animal behaviour and welfare, 4th edition, Broom, D.M. and A.F. Fraser (Eds.), p.65, Cambridge University Press, London.

Chen, S., S. Tanka, C. Oyakawa, S. Roh and S. Sato (2014) Individual difference in serum oxytocin concentrations of calves and the correlation with those in dams. Animal Science Journal, 85: 53–57.

Chen, S., S. Tanaka, S. Ogura, S. Roh and S. Sato (2015) Effect of suckling systems on serum oxytocin and cortisol concentrations and behavior to a novel object in beef calves. Asian-Aus. J Anim. Sci, 28: 1662–1668.

Da, C.A., R.G. Guevara-Guzman, S. Ohkura, J.A. Goode and K.M. Kendrick (1996) The role of oxytocin release in the paraventricular nucleus in the control of maternal behaviour in the sheep. Journal of Neuroendocrinology, 8: 163–177.

Dhakar, M.B., M.E. Rich, E.L. Reno, H.J. Lee and H.K. Caldwell (2012) Heightened aggressive behavior in mice with lifelong versus postweaning knockout of the oxytocin receptor. Hormones & Behavior, 62: 86–92.

Dunbar, R. I (2008) The social role of touch in humans and primates: behavioural function and neurobiological mechanisms. Neuroscience & Biobehavioral Reviews, 34: 260–268.

Holt-Lunstad, J., W.A. Birmingham and K.C. Light (2008) Influence of a "warm touch" support enhancement intervention among married couples on ambulatory blood pressure, oxytocin, alpha amylase, and cortisol. Psychosomatic Medicine, 70: 976–985.

Kondo, S., N. Kawakami, H. Kohama and S. Nishino (1984) Changes in activity, spatial pattern and social behavior in calves after grouping. Applied Animal Ethology, 11: 217–228.

Kurosawa, M., T. Lundeberg, G. Agren, I. Lund and K. Uvnäs-Moberg (1996) Massage-like stroking of the abdomen lowers blood pressure in anesthetized rats: influence of oxytocin. Journal of the Autonomic Nervous System, 56: 26–30.

Mench, J.A., J.C. Swanson and W.R. Stricklin (1990) Social stress and dominance among group members after mixing beef cows. Canadian Veterinary Journal La Revue Veterinaire Canadienne, 70: 345–354.

National Agriculture and Food Research Organization (NARO) (2006) Japanese Feeding Standard for Dairy Cattle. Japan Livestock Industry Association, Tokyo (in Japanese).

National Agriculture and Food Research Organization (NARO) (2008) Japanese Feeding Standard for Beef Cattle. Japan Livestock Industry Association, Tokyo (in Japanese).

Neumann, I., E. Koehler, R. Landgraf and J. Summy-Long (1994) An oxytocin receptor antagonist infused into the supraoptic nucleus attenuates intranuclear and peripheral release of oxytocin during suckling in conscious rats. Endocrinology, 134:141–148.

Ross, H.E. and L.J. Young (2009) Oxytocin and neural mechanisms regulating social cognition and affiliative behavior. Frontiers in Neuroendocrinology, 30: 534-547.

Sato, S., S. Sako and A. Maeda (1991) Social licking patterns in cattle (*Bos taurus*): influence of environmental and social factors. Applied Animal Behaviour Science, 32: 3–12.

Svanidze, M.D., N.G. Bukiya and M.P. Butskhrikidze (2012) Effect of oxytocin on the emotional state and behavior of rats under stress conditions. Neurophysiology, 43: 365–368.

Uvnäs-Moberg, K (1998) Oxytocin may mediate the

benefits of positive social interaction and emotions. Psychoneuroendocrinology, 23: 819–835.
Williams, G.L., O.S. Gazal, L.S. Leshin, R.L. Stanko and L.L. Anderson (2001) Physiological regulation of maternal behavior in heifers: roles of genital stimulation, intracerebral oxytocin release, and ovarian steroids. Biology of Reproduction, 65: 295–300.
Winslow, J.T. and T.R. Insel (1991) Social status in pairs of male squirrel monkeys determines the behavioral response to central oxytocin administration. Journal of Neuroscience the Official Journal of the Society for Neuroscience, 11: 2032–2038.
Winslow, J.T., N. Hastings, C.S. Carter, C.R. Harbaugh and T.R. Insel (1993) A role for central vasopressin in pair bonding in monogamous prairie voles. Nature, 365: 545–548.

Nutritional Characteristics of Forbs and Tree Leaves and Their Contribution to Animal Production in Species-rich Vegetation

Shin-ichiro OGURA[1]*, Hayato MIZUNO[1], Shinta TAKAMIZAWA[1], Masato YAYOTA[2] and Kensuke KAWAMURA[3]

[1]Graduate School of Agricultural Science, Tohoku University, Japan
[2] Faculty of Applied Biological Science, Gifu University, Japan
[3]Japan International Research Center for Agricultural Sciences, Japan

***Corresponding author: Shin-ichiro OGURA**
Tel & Fax +81-229-84-7378
e-mail: shin-ichiro.ogura.e1@tohoku.ac.jp

Keywords: amino acids, grazing ruminants, mineral, nutrient uptake, species diversity

Abstract

Species-rich ecosystems provide mankind with multiple functions that include supporting, provisioning, regulating, and cultural services. In livestock-based grassland ecosystems, however, the effect of plant species richness on food production is less understood. Nutrient composition of plants varies between species, and access to a wider range of plant species by animals increases their foraging choices. In species-rich grasslands, grazing animals encounter and consume a wide range of plant species; therefore, this likely affects the amounts and the proportions of their dietary nutrient intake. To this end, we reviewed several grazing experiments on beef cows in paddocks with varying plant species composition to study the foraging behavior, botanical composition of diets in the cows, and chemical composition of plants. Furthermore, we estimated the concentrations of nutrients in the diet and the amount of nutrient intake per cow. The results showed that 1) the number of plant species foraged by grazing animals rose as species richness of the vegetation increased, 2) the chemical composition of nutrients greatly differed between plant species, with the concentration of some mineral elements (Ca, Mg, Mn, Co, and Se) being higher in some forbs and tree leaves than grasses, 3) the concentration of amino acids was also high in some forbs than grasses and tree leaves, and that 4) higher amounts of nutrient uptake by cows were estimated in species-rich paddocks than in grass dominant pasture. These results suggest that species-rich vegetation including forbs and trees, which are rich in mineral elements and amino acids, improves the nutrient balance of grazing animals.

1.Introduction

Species-rich ecosystems provide mankind with multiple functions, including as supporting, provisioning, regulating, and cultural services (Millennium Ecosystem Assessment 2005). Grasslands ecosystems (permanent pastures and meadows), in particular, cover approximately 25.5% of land area worldwide (FAO 2016) and support livestock production, provide wildlife habitats and recreational lands, and help regulate watersheds (*e.g.*, WalisDeVries *et al*. 1998; Willms and Rode 1998; Shimoda 2010).

In Japan, 65% of land area is covered with forest, and grasslands account for approximately 2.2%. This small proportion of the land area provides habitats for unique wild animals (Sugiura 2004). *Miscanthus sinensis* (Japanese plume grass) and *Zoysia japonica* (Japanese lawn grass) are two of the major species that dominate in these grasslands, and both have long served as cattle grazing pasture (Ogura *et al*. 2004). In addition, these native grasslands contribute an important role in biodiversity conservation. However, these native plants are regarded as low-quality forage by livestock farmers due to their low content of total digestible nutrients and crude protein relative to temperate sown grasses (NARO 2009).

Grazing animals create heterogeneous vegetation owing to the uneven distribution of plants, soil, water resources, topography, and accumulation of excreta (Vallentine 2000). Under these conditions, grazing animals must forage for and select a diet from a range of available foods to meet their nutrient requirements (Forbes 1995). Such diet selection by herbivores can have profound effects on the uptake of nutrients (*e.g.,* sugars, protein, and minerals), plant secondary metabolites, and toxins (Vallentine 2000; Rook and Tallowin 2003; Ogura *et al*. 2006). It can also affect temporal change of vegetation, biodiversity, and sustainable use of the land. Therefore, studies on diet selection and foraging behavior of grazing animals are important for improving livestock productivity and health, and for sustainable management of biodiversity and grassland ecosystems (Ogura 2011).

In species-rich grasslands, grazing animals encounter and consume a wide range of plant species (Ogura 2011). This likely affects the amount and the proportion of the dietary nutrient uptake by grazing animals, taking into consideration that the nutrient composition of plants varies between plant species (NARO 2009). For instance, a study by Ohlson and Staaland (2001) showed that the mineral content of plants greatly varied between plant species (i.e., monocots, forbs, and trees) in habitats of wild moose in north Europe. The authors concluded that the diversity of plant species increases the diversity of mineral elements, and that feeding on few plant species may result in nutrient deficiency or even toxicity in animals. They also assumed that it is necessary for large herbivores in boreal and temperate regions to feed on a wide range of plants to obtain essential minerals in sufficient amounts and in physiologically balanced proportions. Therefore, these previous findings suggest that there are species-specific patterns for nutrient composition in plants, particularly for minerals. Nonetheless, there is lack of knowledge about the intake and balance of amino acids, which likely also affect animal health and productivity (Richardson and Hatfield 1978; O’Connor *et al*. 1993).

We hypothesized that species-rich vegetation enables grazing animals to obtain sufficient essential minerals and amino acids with well-balanced proportions by ingesting forbs and tree leaves. To validate this hypothesis, we reviewed recent studies conducted in species-rich grazing areas in northeastern region of Japan, and discussed the impact of species-rich vegetation on nutrient uptake by grazing animals.

2. Diet composition and nutrient uptake of grazing cattle in species-rich vegetation

Several studies have investigated the effects of plant species-richness in native pastures and young tree plantations on the diet of grazing animals as reviewed by Ogura (2011), which showed a positive relationship between the number of plant species in the diet and that in the vegetation. The Field Science Center (FSC), Graduate School of Agricultural Science, Tohoku University (Osaki, Miyagi, Japan) (38°44′N, 140°15′E) has been one of the major experimental sites of these grazing experiments because of its contrasting vegetational characteristics. Indeed, its many grazing areas consist of orchard grass (*Dactylis glomerata*) dominant pasture, native grasses (*Miscanthus sinensis* and *Zoysia japonica*) dominant pasture, deciduous forest, and cedar forest. Thus, the FSC is an important resource for studying the impacts of plant species richness on nutrient uptake by grazing animals. This is the reason why grazing studies were carried out in the FSC to investigate the effect of plant species richness on nutrient uptake by grazing cattle.

Mizuno *et al*. (2012) assessed the number of plant species in the vegetation and the diet of cattle under three grazing areas with different plant species richness (high: 90, medium: 52, low: 19 species). They showed that the number of species in the diet of cattle was 52–56, 30–35, and 16–17 species in high, medium, and low species richness area, respectively. In the high species richness area, the proportion of dicotyledons consumed by cows was the highest (37.2%) compared to medium (14.8%) and low (10.0%) species richness areas. Takamizawa *et al*. (2016a) found that grazing cows consumed 25–33 plant species in a species-rich mountainous grazing area that comprises 80–85 plant species. This study also showed that the proportions of shrubs and trees consumed were 22.5–44.6% and 6.6–22.8% in summer and autumn, respectively. Their results are indicative that among species-rich vegetation comprising monocots, forbs and trees, forbs and tree leaves constitute over 50% of the diets of the animals, particularly in summer.

Nutrient composition greatly varies between plant species and taxon groups. For example, legumes contain higher crude protein, Ca, and Mg, and lower fiber content than grasses (NARO 2009). Recent studies conducted in the FSC have also revealed high content of Ca and Mn in tree leaves such as *Acer* sp.

and *Weigela hortensis* (Mizuno *et al*. 2012, 2014). The content of Se was relatively high in some native grasses and trees (*e.g.,* dwarf bamboo and *Acer* sp.) (Mizuno *et al*. 2014; Takamizawa *et al*. 2016b). Total amino acid content was high in forb species such as *Rumex acetosella* and *Viola grypoceras* relative to monocots and tree leaves (Takamizawa *et al*. 2016b). On the other hand, tyrosine content was higher in *R. acetosella* (14.0 g kg^{-1} DM) than in monocots (1.0–3.0 g kg^{-1} DM) (Takamizawa *et al*. 2016b).

The differences in nutrient composition between plant species mean that nutrient uptake by grazing animals depends on botanical composition of the diet. As suggested by Ohlson and Staaland (2001), feeding on few plant species may result in a deficient or even toxic nutritional condition. In contrast, feeding on many plant species and taxon groups can result in well-balanced nutritional status (Yoshihara *et al*. 2013). Recent field studies supported these hypotheses. For example, Mizuno *et al*. (2012) showed that the nutrient composition in diet of cows exceeded standard nutritional requirements for K, Ca, Mn, and Zn under grazing in high and medium plant species-richness. Takamizawa *et al.* (2016a) also studied the nutrient content in the diet of grazing cows under species-rich vegetation (*i.e.,* using foraging behavior such as bite size, bite rate, foraging time in pasture and forest, and biting frequency of individual plant species) and nutrient composition of individual plant species. They estimated the nutrient uptake by cows grazing on species-rich vegetation or those on only two monocots (*Anthoxanthum odoratum* and *Carex albata*), and found that the concentrations of Ca, Mg, and Co in animal diets were 17–49% higher under species-rich vegetation than the latter. These results suggest that species-rich vegetation, including monocots, forbs, and trees improve the nutrient balance of grazing animals due to the contribution of forbs and tree leaves that have high concentration of minerals and amino acids.

3. Comparison of nutrient uptake by grazing cattle between in species-rich vegetation and in sown grass pasture

The contribution of forbs and tree leaves to the nutrition of animals was assessed by Takamizawa *et al.* (2016b) by grazing the animals in a sown grass pasture (SP, 1.0 ha) and a combination of sown pasture (0.8 ha) and forest (0.9 ha) area (PF, 1.7 ha). The diet composition and nutrient uptake by cattle were estimated. Orchard grass was the most dominant plant species both in SP (51.8% in coverage) and the pasture area of PF (48.2% in coverage). In the forest area of PF, the coverage of monocots, forbs, trees, and ferns were 17.9%, 22.2%, 24.3%, and 13.1%, respectively. The cows grazed 4.5 and 28.5 plant species in average in SP and PF, respectively. Biting frequency was the highest in orchard grass (75.0–89.2%). In contrast, in the forest area of PF, 21.0 plant species were grazed (48.6% monocots, 29.1% forbs, 20.9% trees, and 1.4% ferns). The plant species also varied in their chemical composition, with Se content being particularly higher in dwarf bamboo (0.10 ppm) than the average content in all plant species (0.03 ppm). In addition, tyrosine content of broad-leaved dock (14.0 g kg^{-1} DM) was more than four times as much as that of monocots (1.0–3.0 g kg^{-1} DM). These nutritional differences between plant species caused significant differences in nutrient content of the diet of grazing cows under SP and PF: *i.e.*, the contents of CP, Ca, Na, Se, Fe, and amino acids in PF cows were 11–78% higher in PF cows than in SP cows. The amount of nutrient uptake (g or mg day^{-1} kg^{-1} body weight) also differed between the vegetation treatments. The daily uptake of Se and tyrosine was 1.6- and 1.3-fold higher in PF cows than SP cows, respectively. This indicates that the combination of a forest area and a sown grass pasture increased the intake of minerals and amino acids by the animals due to the contribution of native grasses, forbs, and tree leaves that have high concentration of these nutrients.

4. Effects of species-richness in the diet on the condition of rumen and blood in grazing cattle

The increase of nutrient uptake (i.e., trace elements and amino acids) by ingestion of forbs and tree leaves in animals may affect nutritional and physiological conditions of the animals. In ruminants, for instance, rumen conditions such as pH, microbial flora and the concentration of nutrients are significantly affected by feed characteristics (VanSoest 1994). This is supported by the findings reported by Mizuno *et al*. (2014), in which ruminal fluids contained more Mn and Se in cattle and sheep grazed in a pasture with high species count (HS) than in low species count (LS). This study also showed differences in bacterial flora between the rumen of animals grazing in HS and those in LS, and that distinctive cellulose-degrading bacteria (*Clostridium saccharolvticm* and *C. phytofermentans*) were detected in HS. These findings suggest that plant species richness affects the digestion and stability of feed in the rumen (Nakano and Ogura 2017).

Changes in species-richness or vegetation composition can also affect the blood condition of animals due to the consequent changes in nutrient composition of the diet. Indeed, Mizuno *et al*. (2012) found that grazing cattle on different plant species affected the mineral concentration in their blood serum, with the concentration of Cu and Se in the blood serum being within the normal range for species-rich grazing area and below the normal range in low species-rich area. This suggests that grazing animal in low species-rich area can result in mineral deficiencies and associated disorders in animals.

4. Implications: the importance of grazing in the species-rich grasslands

Species-rich vegetation provides increased nutrient composition for grazing animals. Therefore, combining use of a sown pasture and a deciduous forest, that creates species-rich vegetation for animals can improve animal health and productivity, in the view that their ingestion of native plants (monocots and forbs) and tree leaves in addition to sown grasses increases the intake of essential minerals and amino acids.

Further studies are needed for further understanding of the role of species-richness in animal diets and, in turn, in the health and productivity of animals. These studies should, however, consider 1) the balance of minerals (i.e., essential elements and toxic metals) and amino acids, 2) rumen digestion characteristics, 3) the long-term effects on animal productivity (e.g., body weight gain, reproduction, and quality of products) and health (e.g., immunity). Such knowledge would also be useful for the sustainable use of species-rich vegetation for herbivore grazing.

Acknowledgments

This study was supported by the Japan Society for the Promotion of Science (JSPS) under KAKENHI Grant number 26292142.

References

Forbes, JM. (1995) Diet selection. In: Voluntary Food Intake and Diet Selection in Farm Animals. CAB International, Wallingford, pp.277–303.

Food and Agriculture Organization (FAO) (2016) FAOSTAT. http://www.fao.org/faostat/en/#home (last access date: 15 December 2016).

Millennium Ecosystem Assessment (2005) Millennium Ecosystem Assessment Synthesis, United Nations, pp.1–219.

Mizuno, H., Y. Yoshihara, T. Inoue, K. Kimura, S. Tanaka, S. Sato and S. Ogura (2012) The effect of species richness of vegetation on mineral condition of grazing cattle in a Japanese alpine pasture. *Proceedings of the 4th Japan-China-Korea Grassland Congress*, pp.242–243.

Mizuno, H., M. Nakano, M. Tohno, K. Kimura and S. Ogura (2014) Rumen conditions of cattle and sheep grazed in pastures with different plant species richness. *Proceedings of the 5th Japan-China-Korea Grassland Congress,* pp.207.

Nakano, M. and S. Ogura (2017) Effect of plant-species richness on microbial composition and rumen function. *Journal of Integrated Field Science,* 14 (in press).

National Agricultural Research Organization (NARO) (2009) Standard tables of feed composition in Japan, pp.25–161.

O'Connor, D. J., C. J. Sniffen, D. G. Fox and W. Chalupa (1993) A net carbohydrate and protein system for evaluating cattle diets: IV. Predicting amino acid adequacy. *Journal of Animal Science,* 71: 1298–1311.

Ogura, S. and K. Sugawara (2004) Grazing use of native pastures by beef cattle in Japan: recent researches on plant-animal interactions. *Tohoku Journal of Agricultural research,* 55: 39–44.

Ogura, S., H. Shinsho, T. Takahashi, M. Obara and K. Sugawara (2006) Heterogeneous impacts of grazing animals and vegetational change in Japanese native pastures. *Journal of Integrated Field Science,* 3: 21–26.

Ogura, S. (2011) Diet selection and foraging behavior of cattle in species-rich vegetation of native grasslands, Japan. *Journal of Integrated Field Science,* 8: 25–33.

Ohlson, M. and H. Staaland (2001) Mineral diversity in wild plants: benefits and bane for moose. *Oikos,* 94: 442–454.

Richardson, C. R. and E. E. Hatfield (1978) The limiting amino acids in growing cattle. *Journal of Animal Science,* 46: 740–745.

Rook, A. J. and J. R. B. Tallowin (2003) Grazing and pasture management for biodiversity benefit. *Animal Research,* 52: 181–189.

Shimoda, K. (2010) Ecosystem Service and Grassland Ecosystem. *Japanese Journal of Grassland Science*, 56: 162–165. (in Japanese only, and title translated by the author)

Sugiura, T. (2004) Grassland as a wildlife habitat. *Grassland Science,* 50: 233–238. (in Japanese only and title translated by the author)

Takamizawa, S., T. Shishido and S. Ogura (2016a) Diet composition and nutrient uptake of cattle in a pasture-forest combining grazing area of northeast Japan. *Proceedings of 10th International Rangeland Congress*, pp.1171–1173.

Takamizawa, S., T. Shishido and S. Ogura (2016b) Effect of native grasses, forbs and trees on nutrient uptake of grazing cattle in a temperate region of Japan. *Proceedings of 10th International Rangeland Congress*, pp.1174–1176.

Vallentine, J. F. (2000) Plant selection in grazing. In: Grazing Management (2nd Edition). Academic Press, San Diego, pp.261–302.

VanSoest, P. J. (1994) Microbes in the gut. In: Nutritional Ecology of the Ruminant (2nd Edition). Cornell University Press, Ithaca, pp.253–280.

WallisDeVries, M. F., J. P. Bakker and S. E. VanWieren (1998) Grazing and conservation management. Kluwer Academic Publishers, Dordrecht, pp.1–363.

Willms, W. D. and L. M. Rode (1998) Forage selection by cattle on fescue prairie in summer or winter. *Journal of Range Management,* 51: 496–500.

Yoshihara, Y., H. Mizuno, S. Ogura, T. Sasaki and S. Sato (2013) Increasing the number of species in a pasture improves the mineral balance of grazing beef cattle. *Animal Feed Science and Technology*, 179: 138-143.

JIFS, 14 : 29 − 31 (2017) Symposium mini review

Seasonal Changes in Forage and Animal Productivity of Korean Native Goats Grazed at Different Forage Type

Se-Young JANG[1], Shin-ichiro OGURA[3], Kensuke KAWAMURA[4], Masato YAYOTA[5],Yeong-Sik YUN[1], Hye-Jin SEONG[1], Sang-Woo KIM[2], Byong-Tae JEON[1] and Sang-Ho MOON[1]

[1]Division of Food Bio Science, Konkuk University, Chungju, 380-701, Korea
[2]Nation Institute of Animal Science, RDA, 590-832, Korea
[3]Graduate School of Agricultural Science, Tohoku University, Japan
[4]Japan International Research Center for Agricultural Sciences, Japan
[5]Faculty of Applied Biological Science, Gifu University, Japan

Corresponding authors: Sang-Ho MOON, moon0204@kku.ac.kr

Keywords: goat, grazing, pasture, seasonal changes

Abstract

This study was conducted to determine grazing intensity of growing Korean native goats (*Capra hircus coreanae*) on mountainous pasture. It was carried out to obtain basic information for improvement of mountainous pasture management and establishing feeding system of Korean native goats. A total of 30 Korean goat were grouped by feeding system [a pasture grazng group (concentrate body weight 1%, treatment 1), a forage grazing group (concentrate body weight 1.5%, treatment 2) and a barn feeding group (TMR, treatment 3), n=10] to conduct study from May to October. Average daily gains (ADG) for the T1, T2 and T3 group were the highest in June for T1 (99.5±6.4d/g), May for T2 (166.7±62.9d/g) and May for T3 (206.7±7.1d/g). The forage productivity of pasture was the largest from May to June (1718.7±207.5~1672.0±422.8g/ha) but it was decreased in July (1356.0±103.8g/ha) because of drought and summer depression. Grazing intensity was calculated by forage productivity and dry matter intake (DMI) and was the highest in May for T1 (65head/ha) and in May for T2 (58head/ha). It is desirable that adequate grazing intensity was maintained by adjusting supplemental feed.

Introduction

The consumption of Korean black goats, which has traditionally been known as a health food, has increased significantly during recent years. The common goat farming system is multiple farming in which large areas of plant resources are available for grazing within fences. And the goats returned to barns where their diet was supplemented with concentrates and this system allows more utilization of grass within the forest (Shahjalal, et al., 1992). Especially, goats differ from other ruminants in their feeding habits, and they appear to have more efficiency in digestion of crude fiber and being better utilizers of poor roughages (Gihad et al, 1980). Hence, goats are highly adaptable with good production potential in the Korea. Geographically, Korea is a mountainous country with 64 percent of its terrain consisting of hills and peaks (Kim, 2002). In addition, Korea having such distinctive four seasons. However, little information is available on the seasonal changes in forage and livestock productivity at different pasture types. The experiment was carried out to obtain basic information for improvement of mountainous pasture management and establishing feeding system of Korean black goats (*Capra hircus coreanae*). Consequently, the objective of this study was to determine grazing intensity of growing Korean native goats on mountainous pasture.

Material and Methods

Animals and diets

Field studies were conducted from the months May to September in order to determine the seasonal forage and animal productivity. Three treatments were selected as experimental farms. Growing goats of farm A (T1) were grazed at pasture and those of farm

B (T2) were grazed at woodland pasture. Comparison group (T3) was a TMR feeding group. All experiment groups has a ten growing wethers of Korean native black goats (*Capra hircus corenae*) aged 4 months. Grazing goats were supplemented by concentrates with T1 (1.0%) and T2 (1.5%). The dry matter yield of pasture and average daily gain of goats were seasonally evaluated in T1 and T2 groups. The forage productivity, dry matter intake (DMI) and grazing intensity were conducted by the method of Moon et al. (2015).

Chemical analysis

Forage samples were analyzed for crude protein (CP), ether extract (EE), crude fiber (CF) and ash according to the methods of the Association of Official Analytical Chemists (AOAC, 1990). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) was analyzed by the procedures of Van Soest et al. (1970).

Statistical analysis

Data were expressed as means and standard errors and were statistically analyzed with Tukey multiple range tests using the SAS package (1955) general linear models (GLM) procedure.

Results and Discussion

Nutrient composition at pasture

The nutrient composition of the experimental pasture is shown in Table 1. Dry matter content was seasonally different at pasture.

Body weight and Average daily gain

Goats of all farms increased consistently body weight. Average daily gain (ADG) of goats were the highest in June (99.5 d/g, T1), in May (127.6 d/g, T2) and in May (206.7 d/g, T3).

Forage productivity, dry matter intake and grazing intensity

The forage productivity was decreased from May to October. The forage productivity of pasture was the highest from May to June (1718.7±207.5~1672.0±422.8 kg/ha) but it was decreased in July (1356.0±103.8 kg/ha) because of drought and summer depression (T1). Grazing intensity was calculated by forage productivity and dry matter intake (DMI) and was the highest in May (65 head/ha, T1) and in May (58 head/ha, T2).

Table 1. Seasonal changes in chemical compositions at different pasture and woodland pasture type grazed by Korea native goats.

Season	Farm	DM[1]	CP[2]	CF[3]	EE[4]	NDF[5]	ADF[6]	Ash
		%	%in DM					
May	T1*	28.75±1.81	17.26±0.11	27.46±0.43	3.40±0.11	60.14±0.47	32.10±0.85	7.92±0.15
	T2**	11.90±0.87	14.94±0.79	22.70±0.62	6.68±0.06	43.99±0.83	32.86±0.80	13.17±0.69
June	T1	33.48±2.56	16.19±0.22	25.22±0.87	4.11±0.33	54.62±0.46	30.92±0.75	7.16±0.15
	T2	19.58±0.12	13.71±0.31	23.11±0.85	6.01±0.27	48.03±0.31	40.25±0.49	12.78±0.26
July	T1	21.24±2.80	19.98±0.61	23.27±0.55	4.32±0.07	58.50±0.71	24.42±0.83	9.09±0.10
	T2	13.32±0.21	19.28±0.69	17.73±0.76	6.82±0.42	41.97±0.59	32.78±0.64	17.52±0.17
August	T1	21.04±2.37	19.77±0.25	26.85±0.35	4.24±0.36	62.41±0.84	29.16±0.19	8.69±0.05
	T2	14.74±0.48	19.11±0.89	29.52±0.84	6.80±0.38	42.91±0.86	30.09±0.78	13.81±0.68
September	T1	20.81±1.57	19.78±0.18	30.25±0.35	4.40±0.03	59.85±0.26	31.96±0.33	8.39±0.05
	T2	19.00±1.35	15.26±0.49	16.39±0.62	7.10±0.87	34.90±0.85	25.44±0.47	21.65±0.13

[1]DM: Dry matter [2]CP: Crude protein [3]CF: Crude fiber [4]EE: Ether extract [5]NDF: Neutral detergent fiber [6]ADF: Acid detergent fiber.
*T1: pasture grazing group **T2: woodland pasture grazing group

Table 2. Seasonal change in average daily gain of Korean native goats grazed at different pasture

	May	June	July	August	September
T1* (d/g)	19.8	99.5	79.1	81.6	37.9
T2** (d/g)	127.6	72.3	20.0	92.6	20.8
T3*** (d/g)	206.7	92.3	112.5	108.3	149.2

*T1: pasture grazing group **T2: woodland pasture grazing group ***T3: TMR grazing group

Table 3. Estimated grazing intensity (head/ha) per ha as affected by forage productivity and dry matter intake at pasture

	May	June	July	August	September	Mean
Forage productivity (kg/ha)	1,718.7 ±207.5	1,672.0 ±422.8	1,356.0 ±103.8	1,280.0 ±153.7	690.7 ±95.9	919.8 ±480.6
Dry matter intake (g/d)	678.4 ±209.9	769.7 ±380.8	606.0 ±1,573.3	689.6 ±1,561.6	808.0 ±1,902.5	770.0 ±170.2
Grazing intensity (head/ha)	65	58	58	48	23	39

Table 4. Estimated grazing intensity (head/ha) per ha as affected by forage productivity and dry matter intake at woodland pasture

	May	June	July	August	September	Mean
Forage productivity (kg/ha)	410.0 ±237.3	460.0 ±20.0	494.7 ±38.0	574.7 ±237.3	506.7 ±498.0	500.9 ±61.41
Dry matter intake (g/d)	650	880	850	870	910	750
Grazing intensity (head/ha)	58	14	15	18	15	28

Conclusion

As shown in the results of this research, grazing intensity was suggested to average 39 head/ha for T1 and 28 head/ha for T2 from May to September. It is desirable that adequate grazing intensity was maintained by adjusting supplemental feed.

Acknowledgement

This work was carried out with the support of the 'Cooperative Research Program for Agriculture Science and Technology Development (Project No. PJ010229)' Rural Development Administration, Republic of Korea.

References

A.O.A.C. (1990) Official methods analysis, Association of official analytical chemists, 15th edition. Kenneth Helrich (Ed.) Association of official analytical chemists, Inc. Arlington, Virginia

Gihad, E. A., T. M. El-Bedawy and A. Z. Mehrez. (1980) Fiber digestibility by goats and sheep. J. Dairy Sci., 63: 1701.

Kim, T.H., K.I Sung. and B.W. Kim (2002) Sustainable livestock production in hill a woodland. Forage production and environment in 21C. : 131–156.

Shahjalal, M., H. Galbraith and J. H.Topps (1992) The effect of changes in dietary protein and energy on growth, body composition and mohair fiber characteristics of British Angora- goats. Anim. Prod, 54: 405–412.

JIFS, 14 : 33－37 (2017)

Effect of Plant-species Richness on Microbial Composition and Rumen Function

Miwa NAKANO[1] and Shin-ichiro OGURA[2]

[1]Institute of Livestock and Grassland Science, NARO, Japan.
Present: Western Region Agricultural Research Center, NARO, Japan.
[2]Graduate School of Agricultural Science, Tohoku University, Japan.

Corresponding authors: Miwa NAKANO, mwnakano@affrc.go.jp

Abstract

Plant diversity has been known to affect the productivity and long-term stability of grassland ecosystem, as well as the mineral balance in grazing cattle. However, there is little information on the relationship between plant diversity and livestock productivity traits such as rumen digestion and fermentation. Rumen bacteria play an essential role in the fermentation and digestion of cattle diet. In microbiology, a highly diverse microbial community is known to be able to respond quickly and flexibly to environmental changes. In fibrous diets-fed cattle, the rumen bacterial diversity is found to be high diverse, as plant-based fibers are rich in complex polysaccharides that enrich the microbial community. Based on the above information, we proposed the following hypothesis: complex fibrous composition of plants and high fiber intake by cattle in native pastures with greater plant species richness lead to high rumen bacterial diversity, which ensures stable fermentation and digestion, as well as flexibility toward rumen environmental changes. This year, we have investigated the rumen bacterial composition profile and rumen digestibility of cows grazing in two pastures with different plant species richness, using molecular biology techniques and in vitro incubation, respectively. Stability will be evaluated in terms of changes in bacterial composition and digestibility after a change in the feeding regime from pasture to barn. The authors believe that understanding the interaction between plant-species richness and livestock productivity will provide not only an evaluation of grassland ecosystem capability, but also the primary knowledge about the maintenance of stable rumen condition for livestock production.

A grassland ecosystem with high plant diversity has been expected to have multiple functions in livestock production. Plant diversity has been known to affect the productivity and long-term stability of a grassland ecosystem (Tilman *et al.*, 2006) as well as plant nutritional characteristics as a feed (Mizuno *et al.*, 2014) and the mineral balance in grazing cattle (Mizuno *et al.*, 2011). However, there is little information on the relationship between plant diversity and livestock productivity traits, such as rumen digestion and fermentation, which are important for livestock production. Microbes, especially bacteria, are essential in rumen function. Bacteria play an essential role in the fermentation of carbohydrates into volatile fatty acids, the degradation of dietary protein, and the partial recapture of non-protein nitrogen as microbial cell protein for subsequent protein nutrition (Mohammed *et al.*, 2014). Therefore, it is necessary to determine rumen bacterial composition as well as its interaction with plant diversity and rumen function—digestion and fermentation—in cattle to evaluate a grassland ecosystem with high plant diversity with respect to livestock production.

A highly diverse microbial community is known to be able to respond quickly and flexibly to environmental changes (Miki, 2011). In rumen microbiology, the rumen bacterial diversity is found to be higher in forage-fed cattle than in high grain diet-fed cattle (Petri *et al.*, 2013). Plant-based fibrous diets are rich in complex polysaccharides (e.g., cellulose and hemicellulose), lignin, and the other phenolic compounds. Fibrous feed particles composed of these compounds are colonized exclusively by closely adherent bacteria (Pitta *et al.*, 2010). Some bacteria specialize in the transformation of plant lignin and the other phenolic compounds and in the formation of secondary substrates, such as cinnamic acid, which enhance polysaccharide hydrolysis by the other members of the biofilm (Larue *et al.*, 2005). These adherent bacteria are often associated with consortia of secondary bacterial colonizers (Pitta *et al.*, 2010). Furthermore, thelong rumen retention time of fibrous diet facilitates the development of a more complex array of diet and intermediary substrates to support a more diverse bacterial community (Pitta *et al.*, 2010). This information suggests that increases in the fibrous nature of the feed particles and the fiber intake of cattle result in greater diversity in the rumen bacterial population, leading to a stable rumen condition that responds quickly and flexibly to rumen environmental changes.

Generally, more plant species are observed in a native pasture compared to those in a sown pasture. Mizuno *et al.* (2012) reported that the number of plant species in pasture vegetation and herbage ingested by cows grazing on a native pasture was higher than that ingested by cows grazing on a sown pasture. The fibrous compound content of herbage ingested by cows grazing on a native pasture is also higher than that of herbage collected on a sown pasture (Nakano *et al.*, 2007; NARO, 2009); seasonal changes in contents of crude protein and neutral detergent fiber of herbage ingested by cows grazing on a native pasture were relatively small (Nakano *et al.*, 2015). The nutritional status (Nakano *et al.*, 2015) and mineral balances (Mizuno *et al.*, 2012) of cows grazing on a native pasture were relatively good, although scant dry matter and energy intake was detected in the fall (Nakano *et al.*, 2015). Based on this information, we hypothesized that ingestion by cattle of a complex fibrous composition of plants owing to the foraging of many fibrous plant species along with the high fiber intake in the native pasture with greater plant-species richness led to high rumen bacterial diversity. This ensures stable rumen function and productivity, flexibility towards rumen environmental changes, and good nutritional status of cattle own.

In preliminary research conducted at the Field Science Center, Graduate School of Agricultural Science, Tohoku University, Japan (38º 44’ N, 140º 15’ E, 300–600 m in elevation) in 2015, the rumen bacterial profiles of cows grazing on two native pastures with medium or high plant-species richness were compared using PCR-denaturing gradient gel electrophoresis (DGGE) of rumen bacterial 16S rRNA gene amplicons. Many bands were visually detected in the profiles of cows grazing on pasture with high plant-species richness compared with those grazing on pasture with medium plant-species richness (Fig. 1). This result suggested that the rumen bacterial population in cows grazing on a pasture with high plant-species richness might be more diverse and characteristic than that observed in cows grazing on a pasture with medium plant-species richness.

The PCR-DGGE method used in this preliminary research allows the rapid screening of bacterial populations and visualization of PCR products representing predominant rumen bacterial communities (Hume *et al.*, 2003). However, this method has to be used in combination with other analyses to identify the bacterial species and to define a detailed population and community structure. Recently, some researchers have studied rumen bacterial populations of cattle fed with high grain diet, high forage diet, silage, and fresh herbage by using next-generation sequencing approaches based on pyrosequencing (Pitta *et al.*, 2010; Mohammed *et al.*, 2014). Petri *et al.* (2013) determined the changes in rumen microbial populations as a result of acidotic inducing diets within individual animals, that have not been well elucidated by using DGGE method, by using 454-pyrosequencing. This approach enables us a new discovery through cost-effective sequence throughput in a relatively short time (Morozova and Marra, 2008). Furthermore, several studies have identified associations between particular rumen bacterial groups and fermentation products in cattle (Hernandez-Sanabria *et al.*, 2010), indicating that the focus in the recent rumen bacterial studies have shifted from determining “who is there” (population) towards understanding “what are they doing” (their mechanisms of action) (Li *et al.*, 2015). The focused correlations among microbial communities in the rumen ecosystems of cattle (Kittelmann *et al.*, 2013) and between bacterial communities and metabolic phenotypes of sika deer (Li *et al.*, 2015) have been described visually by using co-occurrence network analysis. Co-occurrence network analysis is generally used to provide a graphic visualization of potential relationships between people, organizations, concepts, or other entities represented within the available information (Yasuda, 1997). In rumen microbiology, this analysis is employed for understanding the metabolic networks between rumen-inhabiting microbial groups and resolving their individual contributions to overall rumen functioning (Kittelmann *et al.*, 2013). This approach may provide new clues about complex interactions among rumen bacterial diversity, rumen function, and its stability in cattle grazing on species-rich pastures.

Using next generation sequencing and co-occurrence network analysis, the objectives of this study were to 1) investigate differences in the fibrous composition of ingested herbage, rumen retention time, and bacterial composition of cattle grazing on pastures with high or low plant-species richness; 2) explore the associations among rumen bacterial diversity, rumen function (rumen digestibility), and productivity (fermentation characteristics and amount of microbial protein synthesis); 3) explore the relationship between rumen bacterial diversity and stabilities of rumen digestion and fermentation; and 4) test the hypothesis that rumen bacterial diversity and rumen function and productivity are related to the plant-species richness in a pasture.

The study has conducted from May 2016 at the Field Science Center, Graduate School of Agricultural Science, Tohoku University, Japan (38º 44’ N, 140º 15’ E, 300–600 m in elevation). The typical rumen bacterial composition profile and rumen digestibility of cows grazing in two pastures with high (native pasture) or low (sown pasture) plant-species richness have been investigated using next-generation sequencing and *in vitro* incubation, respectively. Stability will be evaluated in terms of changes in bacterial composition and digestibility after a change in the feeding regime from pasture to barn. The authors have collected rumen fluid, urea, and blood samples from cows grazing on native and sown pastures, and has analyzed elements associated with rumen bacterial diversity, rumen fermentation, bacterial cell protein synthesis, and nutritional status. The authors hope to obtain interesting discoveries and discuss with you in other opportunities.

Recently, in the dairy and beef production industry, rumen acidosis is increasingly recognized as a significant disorder. Rumen acidosis results from extreme fermentation due to feeding of high grain and low roughage diets. This extreme fermentation leads to an increase in acid and a decline in pH in the rumen, which alters the activity and abundance of many bacterial species (Petri *et al.*, 2013). Rumen fermentation and bacterial population during rumen acidosis have been extensively studied (Petri *et al.*, 2013; Mao *et al.*, 2013). Nevertheless, it is also necessary to reveal the stable rumen condition—bacterial composition and rumen function—that can adapt flexibly toward these rapid changes in feed. An understanding of the interaction between plant-species richness and livestock productivity would lead to an understanding of the factors responsible for stable rumen condition. The authors believe that understanding the interaction between plant-species richness and livestock productivity will provide not only an evaluation of grassland ecosystem capability, but also primary knowledge about the maintenance of stable rumen condition for livestock production.

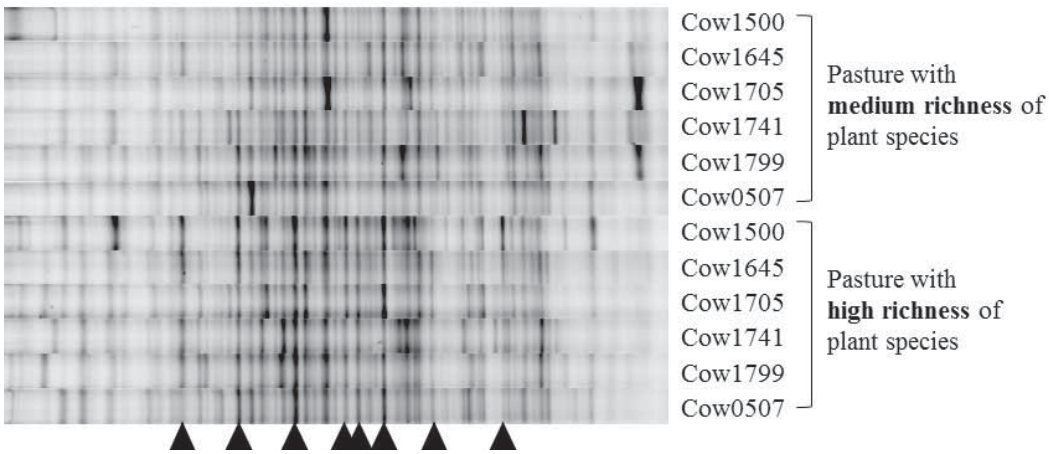

Fig. 1. Denaturing gradient gel electrophoresis (DGGE) profiles of rumen bacterial 16S rRNA gene amplicons from cows grazing on two native pastures with medium or high plant-species richness in the preliminary research conducted in 2015. The image was normalized using the DGGE Marker II (Nippongenem Toyama, Japan) and Bio Numerics software, version 5.0 (Applied Maths, Austin, TS). Filled triangles (▲) under the figure indicate the bands that were detected strongly or characteristically in pasture with high plant-species richness.

Acknowledgement

The authors are grateful to T. Shishido, T. Chiba, S. Takamizawa, Dr. A. Tozawa, R. Futahashi, N. Hatakeyama, QIANRIGE, Yin Liying (Tohoku University), Dr. Y. Yamamoto, Dr. Y. Togamura, Dr. H. Ishizaki, and Dr. S. Haga (NARO, institute of Livestock and Grassland Science) for their useful suggestions and their technical assistance. This work was supported by JSPS KAKENHI Grant Number JP16K18789.

References

National Agriculture and Food Research Organization (NARO) (2009) Standard tables of feed composition in Japan. Japan Livestock Industry Association, Tokyo, Japan. (in Japanese)

Hernandez-Sanabria, E., L.L. Guan, L.A. Goonewardene, M. Li, D.F. Mujibi, P. Stothard, S.S. Moore and M.C. Leon-Quintero (2010) Correlation of particular bacterial PCR-denaturing gradient gel electrophoresis patterns with bovine ruminal fermentation parameters and feed efficiency traits. Appl Environ Microbiol, 76: 6338–6350.

Hume, M.E., L.F. Kubena, T.S. Edrington, C.J. Donskey, R.W. Moore, S.C. Ricke and D.J. Nisbet (2003) Poultry digestive microflora biodiversity as indicated by denaturing gradient gel electrophoresis. Poult Sci, 82: 1100–1107.

Kittelmann, S., H. Seedorf, W.A. Walters, J.C. Clemente, R. Knight, J.I. Gordon and P.H. Janssen (2013) Simultaneous amplicon sequencing to explore co-occurrence patterns of bacterial, archaeal, and eukaryotic microorganisms in rumen microbial communities. PLoS ONE, 8: e47879.

Larue, R., Z. Yu, V.A. Parisi, A.R. Egan, and M. Morrison (2005) Novel microbial diversity adherent to plant biomass in the herbivore gastrointestinal tract, as revealed by ribosomal intergenic spacer analysis and rrs gene sequencing. Environ Microbiol, 7: 530–543.

Li, Z., A.G. Wright, H. Liu, K. Bao, T. Zhang, K. Wang, X. Cui, F. Yang, Z. Zhang, and G. Li (2015) Bacterial community composition and fermentation patterns in the rumen of sika Deer (*Cervus nippon*) fed three different diets. Microb Ecol, 69: 307–318.

Mao, S.Y., R.Y. Zhang, D.S. Wang, and W.Y. Zhu (2013) Impact of subacute ruminal acidosis (SARA) adaptation on rumen microbiota in dairy cattle using pyrosequencing. Anaerobe, 24: 12–19.

Miki, T (2011) Microbial Population Biology. In Microbial Ecology, T. Ohsono and M. Kagami (Eds.), pp. 222–237, Ecological Society of Japan, Tokyo. (in Japanese)

Mizuno, H., M. Nakano, M. Tohno, K. Kimura and S. Ogura (2014) Rumen conditions of cattle and sheep grazed in pasture with different plant species richness. Knowledge innovation of grassland science and sustainable development of grassland agriculture. Proceedings of the 5th China-Japan-Korea Grassland Conference, p. 203.

Mizuno, H., Y. Yoshihara, T. Inoue, K. Kimura, S. Tanaka, S. Sato and S. Ogura (2012) The effect of species richness of vegetation on mineral condition of grazing cattle in a Japanese alpine pasture. Evolution and future challenges of grasslands and grassland agriculture in the East Asia. Proceedings of the 4th Japan-China-Korea Grassland Conference, pp. 242–243.

Mohammed, R., G.E. Brink, D.M. Stevenson, A. P. Neumann, K.A. Beauchemin, G. Suem and P.J. Weiner (2014) Bacterial communities in the rumen of Holstein heifers differ when fed orchardgrass as pasture vs. hay. Front Microbiol, 5: 1–11.

Morozova, O. and A.M. Marra (2008) Applications of next-generation sequencing technologies in functional genomics. Genomics, 92: 255–264.

Nagaraja, T.G. and K.F. Lechtenberg (2007) Acidosis in feedlot cattle. Veterinary Clinics of North America. Food Anim Pract, 23: 333–350.

Nakano, M., M. Yayota and S. Ohtani (2015) Nutrient sufficiency in beef cows grazing on a dwarf bamboo-dominated forestland pasture in Japan. Anim Sci J, 86: 689–697.

Nakano, M., M. Yayota, J. Karashima and S. Ohtani (2007) Seasonal variation of nutrient intake and digestibility of forage in beef cows grazed on a dwarf bamboo (*Pleioblastus argenteostriatus f. glaber*) dominant pasture. Grassl Sci, 53: 69–77.

Petri, M.R., T. Schwaiger, G.B. Penner, K.A. Beauchemin, R.J. Forester, J.J. McKinnon and T.A. McAllister. (2013) Characterization of the core rumen microbiome in cattle during transition from forage to concentrate as well as during and after an acidotic challenge. PLoS ONE, 8: e83424.

Petri, R.M., R.J. Forster, W. Yang, J.J. McKinnon and

T.A. McAllister (2012) Characterization of rumen bacterial diversity and fermentation parameters in concentrate fed cattle with and without forage. J Appl Microbiol, 112: 1152–1162.

Pitta, D.W., W.E. Pinchak, S.E. Dowd, J. Osterstock, W. Gontcharova, E. Youn, K. Dorton, I. Yoon, B.R. Min, J.D. Fulford, T.A. Wickersham and D.P. Malinowski (2010) Rumen bacterial diversity dynamics associated with changing from bermudagrass hay to grazed winter wheat diets. Microb Ecol, 59: 511–522.

Tilman, D., P.B. Reich and J.M. Knops (2006) Biodiversity and ecosystem stability in a decade-long grassland experiment. Nature, 441: 629–632.

Yasuda, Y (1997) Network analysis. Y. Yasuda (Ed.), pp. 1-256, Shinyosya, Tokyo, Japan.

Monitoring Foraging Behavior in Ruminants in a Diverse Pasture

Masato YAYOTA[1]*, Kazuya DOI[2], Kensuke KAWAMURA[3] and Shin-ichiro OGURA[4]

[1]Faculty of Applied Biological Sciences, Gifu University, Gifu, Japan
[2]The United Graduate School of Agricultural Science, Gifu University, Gifu, Japan
[3]Japan International Research Center for Agricultural Sciences, JIRCAS, Tsukuba, Japan
[4]Graduate School of Agricultural Science, Tohoku University, Sendai, Japan

***Corresponding Author: Masato Yayota**
Tel & Fax: +81-58-293-2867
e-mail: yayo@gifu-u.ac.jp

Keywords:automatic sensors, diverse pasture, foraging behavior, grazing animals, monitoring

Abstract

The foraging behavior of grazing ruminants plays an important role in their production as well as in maintaining plant diversity. The effects of plant species richness on animal production, health, and welfare occur only after animals have foraged on the relevant plant species. Thus, monitoring foraging behavior is a key tool for understanding the underlying relationships between plant species richness and animal production, health, and welfare. Efforts to monitor foraging behavior have, however, been confronted with methodological difficulty due to the complex and labor-intensive nature of the monitoring process. This difficulty increases when ruminants encounter more heterogeneous and diverse feeding environments, such as semi-natural or forest pastures, because they choose and ingest various plant species that have different structures and morphologies. Recent developments in information and communication technology will drastically change this situation. Compact and wearable devices, such as acceleration sensors, can detect complex and fine animal movements precisely over a relatively long period. In this review, we first summarize the foraging process in grazing ruminants to provide general knowledge for monitoring foraging behavior. Second, we briefly review previous findings from studies that have monitored foraging behavior using automatic sensors and consider the foraging behavior of animals when they ingest plants whose forms are different from those of typical short grasses. Third, recent findings on foraging behavior in diverse feeding environments that were revealed using a new technique are presented. Finally, the remaining issues regarding monitoring and assessing the foraging behavior of ruminants in diverse feeding environments are discussed.

1. Introduction

The foraging behavior of grazing ruminants plays an important role in their production, health and welfare states as well as in grassland management, including its productivity, sustainability and diversity. In homogeneous grasslands such as sown pastures, animal performance as a corollary of foraging behavior is almost predictable because we can estimate the nutritional composition of what the animals eat relatively easily. However, as stated in Ogura *et al*. (2017) on this issue, when animals graze on heterogeneous or species-rich grasslands, they have an opportunity to choose and ingest a variety of plant species that have different nutrient compositions. This situation leads to a hypothesis that the wider range of diet choice will provide animals with balanced nutrients. In fact, recent studies have suggested that great richness of plant species improved the intake of energy, protein (Wang *et al*. 2010) , minerals and amino acids (Yoshihara *et al*. 2013; Ogura *et al*. 2017) and may influence ruminal fermentation and digestion via changes in the microbial flora (Nakano and Ogura 2017). These changes are expected to have positive effects on animal production, health, and welfare. However, it should be emphasized that all of these results occur only after animals have foraged on the relevant plant species. Thus, monitoring foraging behavior is a key tool for understanding the underlying relationships between plant species richness and animal production, health, and welfare.

Efforts to monitor foraging behavior have, however, been confronted with methodological difficulties. The direct observation of foraging behavior is laborious, time-consuming and often implemented under unpleasant conditions (Penning and Rutter 2004). In general, a foraging bout lasts for about one to two hours, and the total daily grazing time ranges from 6 to 12 hours. This leads to a decrease in the concentration of observers and an increase differences in the interpretation of foraging activities among individuals. Another difficulty is that foraging behavior is composed of continuous and fine-scale jaw movements, and animals choose and eat one plant species after another. The processes of choice and ingestion via fine jaw movements occur within seconds. Thus, direct observation may be impossible for some foraging parameters (Penning and Rutter 2004).

These difficulties have promoted the development of many automatic monitoring devices and classification algorithms over recent decades. These efforts have successfully allowed the monitoring of foraging behavior and have advanced the understanding of fine foraging processes in animals in homogenous feeding environments (e.g., Laca *et al*. 1992a; Laca and Wallis De Vries 2000; Galli *et al*. 2011). However, the difficulty increases when ruminants graze in more heterogeneous and diverse feeding environments such as semi-natural or forest pastures because they choose and ingest various plant species that have different sizes, structures and morphologies. This complex situation causes observers to have to detect and identify the fine jaw movements used for various plant species instantaneously.

Recent developments in information and communication technology (ICT) will drastically change this situation. Compact and wearable devices such as acceleration sensors, action cameras and miniature data loggers can detect and record complex and fine animal movements precisely over a relatively long period. On the other hand, direct observation still has advantages in terms of flexibility and adaptability (Penning and Rutter, 2004; Bonnet *et al*. 2016). Therefore, combining direct observation and automatic devices is a feasible technique for monitoring and analyzing the foraging behavior of grazing ruminants in diverse pastures.

In this review, we first summarize the foraging process of grazing ruminants to provide general knowledge for monitoring foraging behavior. Second, we briefly review previous findings from studies that have used automatic sensors to monitor foraging behavior and consider the foraging behavior of animals when they ingest plants whose forms are different from those of typical short grasses. Third, recent findings on foraging behavior in diverse feeding environments that were revealed using a new technique are presented. Finally, the remaining issues regarding monitoring and assessing the foraging behavior of ruminants under diverse feeding environments are discussed.

2. Foraging process of grazing ruminants

Conceptually, the foraging behavior of ruminants has a temporally and spatially hierarchical structure (Senft *et al*. 1987; Bailey *et al*. 1996). The basic component of foraging or grazing behavior is a bite. The bite involves prehending and cutting a bunch of forage from a grassland (Ungar 1996). Ruminants bite some plants or plant parts at a feeding station, which is defined an array of plants available to an herbivore without moving their front feet (Novellie 1978; Ruyle and Dwyer 1985); they then move to a new feeding station. A cluster of feeding stations is called a patch. A patch is also identified by a break in the foraging sequence. In the same manner, a cluster of patches is defined as a feeding site. At a feeding site, animals continuously graze plants during a foraging bout. A set of feeding sites is called a camp, where animals drink and rest between foraging bouts. The top of the foraging hierarchy is the home range, which is defined by fences, barriers, the extent of migration, or transhumance (Bailey *et al.* 1996). When monitoring the foraging behavior of ruminants, the grazing process at a feeding station is critical because it falls on the very bottom of the foraging hierarchy.

At a feeding station, animals search for plants or plant parts according to their characteristics, such as quantity, quality, architecture and morphology. They then bite the forage—prehend a bunch of forage using the tongue, gums and lower jaw—and cut the herbage with a sudden movement of the lower jaw and the whole head (Ungar 1996; Andriamandroso *et al*. 2016). The ingested forage is ground using the molar teeth. This process is called chewing or mastication. Interestingly, some ruminants, such as cattle, sheep and goats, perform these two processes—biting and chewing—simultaneously (Laca and Wallis De

Vries., 2000; Milone *et al*. 2009; Navon *et al*. 2013). This ingestive behavior is defined as chew-bites (Laca and Wallis De Vries. 2000). Finally, they swallow the forage into the esophagus. All these processes are not mutually exclusive (Ungar 1996) (Fig. 1).

The bite can be divided into further components (Fig. 1). When animals prehend and remove a bunch of forage in one bite, the total volume of the forage is defined as the bite volume (Laca *et al*. 1992a; Ungar 1996). The bite volume is the product of the bite area and bite depth. The bite area is the harvested area taken by an animal in one bite, and the bite depth is the difference between the initial and residual height of the same plant(s) before and after grazing (Laca *et al*. 1992a; Ungar 1996). The bite mass (or bite weight) is the product of the bite volume and the weight of forage per unit volume in that space (Allen *et al*. 2011).

Plant characteristics such as size and architecture affect the bite dimensions: bite area, bite depth, and bite volume (Laca *et al*. 1992b; Ungar 1996; Searle and Shipley 2008). These bite dimensions determine the bite mass. Similarly, plant characteristics influence the cropping time, and this cropping time and the chew rate are involved in the rate of bites (bite rate) (Ungar 1996). The bite mass and bite rate are the factors that determine the intake rate, and the product of the intake rate and grazing time is the daily intake (Penning and Rutter 2004; Carvalho 2013). Daily intake directly relates to animal performance and health on one hand, and the daily intake linked with the movement patterns of animals affects the spatial heterogeneity of the landscape and grassland diversity (Searle and Shipley 2008). Considering this hierarchical foraging process (Fig. 2), bite mass and bite rate are the relevant properties in monitoring ruminant foraging behavior.

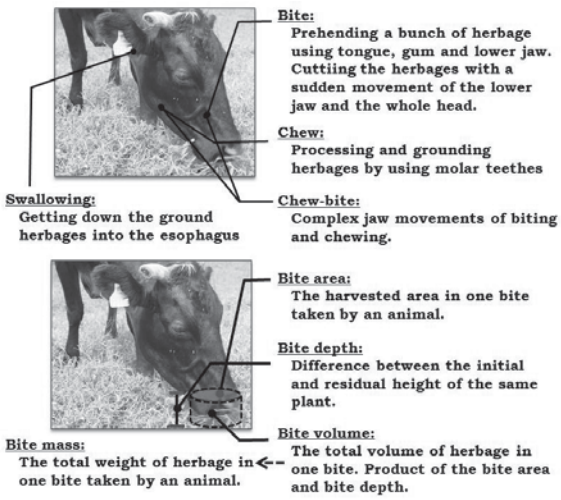

Fig. 1. Grazing jaw movements and bite dimensions of ruminants at a feeding station. (Terminologies are defined by Laca et al. (1992a), Ungar (1996) and Allen *et al*. (2011).)

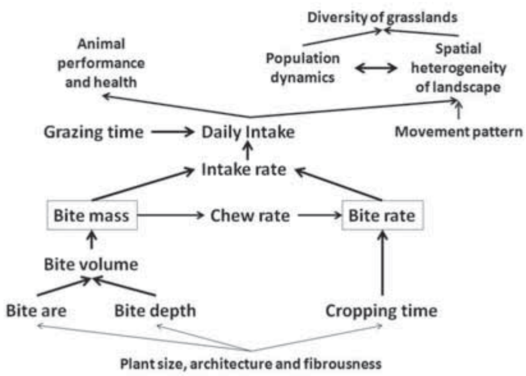

Fig. 2. Hierarchical relationships between plant characteristics, foraging behavior, daily intake, animal performance and the grassland ecosystem (adapted from Ungar (1996), Sealr and shipley (2008), and Carvalho (2013)).

3.Monitoring foraging behavior of grazing ruminants using automatic sensors

The problem is how we assess these fine jaw movements over the course of a day or, at least, a foraging bout. During the last five decades, many researchers have developed monitoring techniques for foraging jaw movements using mechanical and electrical sensors. There have been several excellent reviews on this topic (Penning and Rutter 2004; Navon *et al*. 2013; Andriamandroso *et al*. 2016); thus, only a brief summary is presented here.

The techniques are roughly divided into 6 categories based on the sensor: pneumatic sensors, transducers, electromyograms, pressure sensors, microphones and acceleration sensors. All of these sensors are attached to the head or the neck of the animal and record the jaw and/or head movements during eating and ruminating. Although what the sensors record slightly differs among the sensors, they principally convert the signals of physical jaw or head movements to waveforms. For example, an acceleration sensor detects static acceleration due to gravity, the low-frequency component of the acceleration and the dynamic acceleration due to movements by an animal (Brown *et al*. 2013). The sensor transforms physical acceleration from motion or gravity into waveforms. Each distinctive pattern in the waveforms shows the occurrence of grazing jaw movements. Thus, we can detect and identify the differences in waveforms among fine jaw movements and determine when and how many of each jaw movement occurred during observation. Some researchers have developed algorithms that can automatically identify types of jaw movements such as bite, chew, and chew-bite (Clapham *et al*. 2011; Milone *et al.* 2012; Chelotti *et al*. 2016).

Many previous studies using these sensors have successfully monitored grazing jaw movements in homogeneous feeding environments, mainly monocultures of short grass swards (e.g., Laca *et al*. 1992a; Rutter *et al*. 1997; Laca and Wallis De Vries. 2000). Although the determination of bite mass by the sensors has been a challenging problem, combining these sensors with a micro-sward technique or other methods has revealed functional relationships among intake rate, bite rate, bite mass and bite dimensions in homogenous swards (Laca *et al*. 1992b, 1994; Ungar 1996; Galli *et al*. 2011).

4.Foraging behavior for different plant forms

There are many plant species that have different sizes, architectures and morphologies. Different plant forms necessitate different motor patterns for their harvest (Flores *et al*. 1989). For example, when lambs graze grasses, they mainly show a typical prehension pattern: gripping grass tillers with their incisors and the upper dental pad while jerking the head forward or backward. However, when lambs graze shrubs, they show more complex prehension patterns that are composed of plucking individual leaves, breaking twigs and stripping leaves off branches (Flores *et al*. 1989).

Hirata *et al*. (2011) assessed the foraging behavior of cattle grazing tall grasses over 1 m in height with full-sized or half-sized leaves. They observed that cattle initially grazed leaves at the lower levels and progressively shifted to leaves at the upper levels of a tall grass. They also found that cattle sometimes ingested one leaf before completely consuming a previously detached leaf (Hirata *et al.* 2011). When cattle ate a half-sized leaf, they prehended the mid or distal part of the leaf. They then ate a leaf by chewing and swallowing part of the leaf while the reminder was attached to the stem. Contrastingly, cattle usually prehended the proximal or mid part of a full-sized leaf and severed the leaf from the stem with a movement of the head. Their results suggested that cattle show different motor patterns when foraging tall grasses and can control the allocation of jaw movements corresponding to vertical and horizontal leaf arrangements.

Moreover, Yayota *et al*. (2015) examined the foraging behavior of cattle when they encounter plants of different sizes and morphologies. They used four test plants: bahiagrass (*Paspalum notatum* Flügge; a short grass), Sudangrass (*Sorghum* × *drummondii*; a typical tall grass) and two growth forms of a dwarf bamboo (*Sasa senanensis*; a semi-woody plant): a naturally growing form (approximately 2 m tall) and a form that is under grazing (<0.8 m tall). The grazing jaw movements were assessed using a one-axis acceleration sensor and a micro-sward technique. Their results show that the total number of jaw movements is relatively the same among the plants (approximately 70 times per minute); however, the components of the grazing jaw movements are different. For example, the cattle used small numbers of bite or chew-bite jaw movements and frequently used chewing jaw movements when they ate dwarf bamboos. In contrast, the cattle frequently used bite and chew-bite movements when they ate the short grass (bahiagrass). Accordingly, the cattle showed a greater bite mass and slower bite rate when they ate the taller dwarf bamboo, whereas they showed a smaller bite mass and faster bite rate when they ate the short grass. These results suggested that the cattle were able to control their ingestive jaw movements depending on the plant characteristics, such as height, the spatial arrangement of the leaves and leaf morphology.

In brief summary, ruminants have the ability to control their ingestive behavior and/or jaw movements depending on the plant size, architecture and leaf morphology. Bite mass and bite rate vary with such ingestive behavior. These results likely suggest that biting style—how forage is prehended and severed—corresponding to plant morphology is the key factor that produces different bite masses and bite rates.

5. Monitoring foraging behavior in diverse pastures

Free-ranging animals continuously choose and ingest different species and types of plants in diverse pastures. In this context, the functional relationships among intake rate, bite rate, bite mass and bite dimension obtained in a homogenous pasture are not applicable because animals continuously change their grazing jaw movements based on the different plant species or types. Thus, we have to monitor the selection of plant species or type and bite style, including bite rate and bite mass, by grazing animals in a different way.

One of the recent promising techniques is the creation of a "bite-coding grid". Fundamental to this technique is the definition of several bite categories depending on the structural differences in the forages. Each bite category is illustrated as a symbolic image, and these categories are grouped by general botanical group and assumed bite mass. The bite-coding grid was originally developed by Agreil and Muret (2004) for small ruminants grazing on shrubby rangelands. Later, some researchers including themselves adapted it to several diverse pastures (Agreil and Muret 2008; Bonnet *et al*. 2011: Gonzalez-Pech *et al*. 2014). Bonnet *et al*. (2015) presented the detailed protocol for making bite-coding grids and introduced an application of the method for grazing animals on a diverse pasture in the Brazilian Pampa. They emphasize the importance of direct observation when making a bite-coding grid and conducting bite monitoring of grazing animals and highlight the problems with video recording: the lack of a three-dimensional perspective and several senses such as color, texture, touch and smell in the environmental context of the local plant community.

However, the direct observation of grazing animals is a time-consuming and labor-intensive process, and we sometimes do not capture foraging behavior using this method due to the fine and rapid movements involved, even if we use trained and tamed animals. We believe that techniques using video recording to design bite-coding grids and monitor foraging behavior are a viable alternative. Thus, we have attempted to develop a bite monitoring technique using a handheld video camera and a wearable camera.

We conducted a study on a semi-natural pasture that used to be an abandoned field for years, at Minokamo City in Gifu, Japan. Sixteen goats were set stocked from mid-May to early November from 2013. The pasture was dominated by graminoids, forbs, and woody plants, including shrub-like bamboos, during the years of the study (Tamiya *et al.* 2016). We identified more than 60 plant species in 2015 and approximately 80 plant species in 2016.

In the first stage, we defined bite categories and designed a bite-coding grid for the goats using a handheld camera (GZ-MG 575, JVC KENWOOD Co. Ltd., Kanagawa, Japan). The foraging behavior of six goats was recorded for at least 30 minutes. All visual assessments were conducted using video editing software (Power- Director Express 13; CyberLink Inc., Tokyo, Japan).

The resulting bite-coding grid in the semi-natural pasture is shown in Fig. 3. According to the similarities in plant structure, leaf morphology and assumed bite mass, 26 bite categories were defined by the video assessments. For example, one of the bite code groups is for graminoids, including Poaceae and Cyperaceae. This group was divided into five bite categories according to the bite depth. Forbs that have erect stems, such as Canadian goldenrod (*Solidago canadensis*) and *Erigeron annuus*, were also divided into five bite categories according to the assumed bite mass. One of unique plants in this pasture was a bamboo (*Phyllostachys edulis* (Carrière) Houz.). Generally, a bamboo is a giant, timber-like plant and reaches approximately 15 m tall, but it maintains semi-woody forms under heavy grazing conditions. Therefore, we decided to make independent bite categories for the bamboo.

According to this bite-coding grid, we assessed the frequency of appearance of each bite category. For this objective, we attached a wearable camera (HX-A500, Panasonic Co. Ltd., Osaka, Japan; MPEG-4 AVC, 1280×720 pixel, flame rates 30p) to the heads of the goats using a halter. The foraging behavior of five goats was recorded for 15 minutes. Of the 26 bite categories in this pasture, we can identify 24 bite types for the goats (Fig. 4). The frequency of appearance of each bite category differed among the individual goats (Fig. 4). This result was mainly caused by the locations where the goats foraged in the pasture because the plants classified into the different bite categories were unevenly distributed in the pasture. In this preliminary study, only three to five goats were analyzed using this technique. If more animals are analyzed, more bite categories will be required to monitor the foraging behavior of the goats.

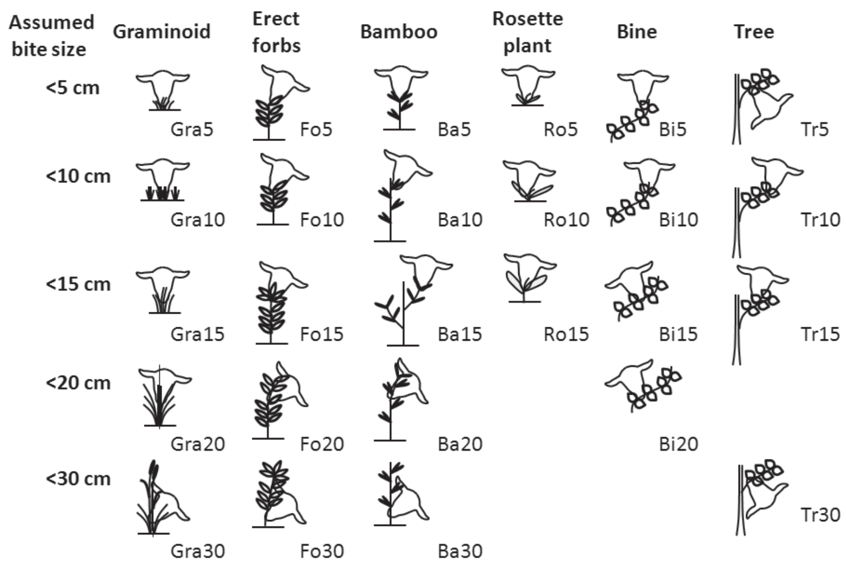

Fig. 3. Bite-coding grid for goats in a semi-natural pasture in Japan. Each pictogram illustrates the typical bite for each bite category. Bite categories are arranged according to plant structural characteristics and assumed bite size.

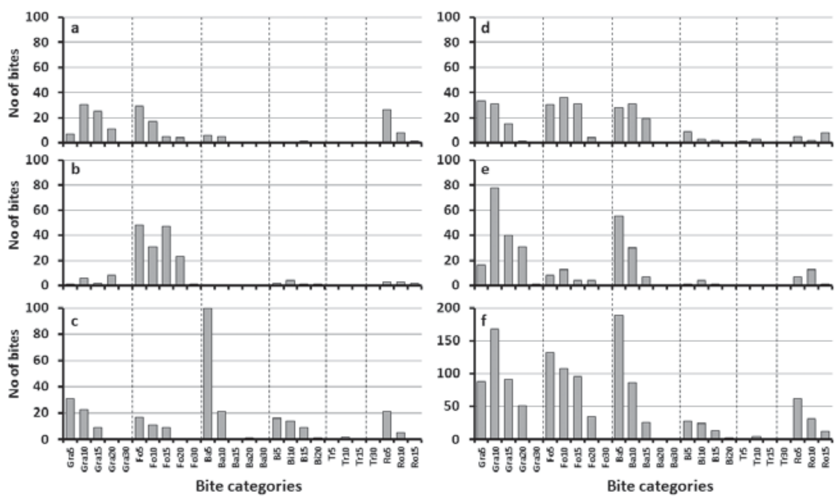

Fig. 4. Frequencies of the observed bite categories during the observation: a–e show the results from individual goats and f shows the total result from the observation. Counts and identification of the frequencies of each bite category were carried out using visual images from a wearable camera. See Fig. 3 for details of the bite categories.

6. Concluding remarks

Overall, we can design a bite-coding grid in a diverse feeding environment using a video recording technique. The use of a wearable camera with a bite-coding grid was successful in monitoring the foraging behavior of a small ruminant. Repeat playback, slow-motion replay and still images are favorable aspects when video recording devices are used; however, the direct assessment of plant species and their structure and the direct observation of animal foraging behavior are important considerations before the use of video recording techniques.

Even if we can successfully monitor the foraging behavior of grazing animals using a bite-coding grid, some issues still remain. One issue is the determination of bite mass in each bite category. As shown in Carvalho *et al*. (2013), some bite categories have a wide range in bite mass. This variation causes the over- or underestimation of bite mass, the short-term intake rate and the resulting daily intake. Another issue is the automatic identification of bite categories. The use of a wearable camera may reduce laborious work in the field; however, the visual assessment of video footage is also a time-consuming process. Currently, multi-axis acceleration sensors, such as six- and nine-axis acceleration sensors, are easily obtained, and the combination of such sensors and machine learning, such as decision forests and neural networks, may allow the characteristic features of each bite category to be detected.

In conclusion, although several challenging problems still remain, by using new wearable sensors and supplementary direct techniques, we can monitor the foraging behavior of grazing animals more precisely. This leads to the measurement of temporal changes in diet selection and nutrient intake in free-ranging animals in diverse feeding environments. The development of these techniques and technologies will be helpful in improving the production, health, welfare, and behavioral enrichment of grazing animals. Furthermore, by knowing when, where, how many, and what types of plant species are ingested by grazing animals, we can understand herbivore-plant interactions more precisely. This will contribute to the development of the multi-function of plant species for animal production, appropriate grassland management and conservation.

Acknowledgments

This study was supported by the Japan Society for the Promotion of Science (JSPS) under KAKENHI Grant number 26292142.

References

Agreil, C. and M. Meuret (2004) An improved method for quantifying intake rate and ingestive behaviour of ruminants in diverse and variable habitats using direct observation. *Small Ruminant Research*, 54: 99–113.

Agreil, C. and M. Meuret (2008) Recording the prehensive bite diversity in cows for a dynamic analysis of foraging behavior on diversified vegetation. *Proceedings of the 21st International Grassland Congress and 8th International Rangeland Congress*, 1: 439.

Allen, VG., C. Batello, EJ. Berretta, J. Hodgson, M. Kothmann, X. Li, J. Mclvor, J. Milne, C. Morris, A. Peeters and M. Sanderson (2011) An international terminology for grazing lands and grazing animals. *Grass and Forage Science*, 66: 2–28.

Andriamandroso, AHL., J. Bindelle, B. Mercatoris and F. Lebeau (2016) A review on the use of sensors to monitor cattle jaw movements and behavior when grazing. *Biotechnolgy Agronomy Society and. Environment*, 20 (S1): 1–14.

Bailey, DW., JE. Gross, EA. Laca, LR. Rittenhouse, MB. Coughenour, DM. Swift and PL. Sims (1996) Mechanisms that result in large herbivore grazing distribution patterns. *Journal of Range Management*, 49: 386–400.

Bonnet, OJF., N. Hagenab, L. Hebbelman, M. Meuret and AM. Shrader (2011) Is hand plucking an accurate method of estimating bite mass and instantaneous intake of grazing herbivores? *Rangeland Ecology and Management*, 64: 366–374.

Bonnet, OJF., M. Meuret, MR. Tischler, IM. Cezimbra, JCR. Azambuja and CF. Carvalho (2015) Continuous bite monitoring: a method to assess the foraging dynamics of herbivores in natural grazing conditions. *Animal Production Science*, 55: 339–349.

Brown, DD., R. Kays, M. Wikelski, R. Wilson and AP. Klimley (2013) Observing the unwatchable through acceleration logging of animal behavior. *Animal Biotelemetry*, 1: 20.

Carvalho, PCF. (2013) Can grazing behavior support innovations in grassland management? *Tropical-Grasslands - Forrajes Tropicales,* 1: 137–155.

Chelotti, JO., SR. Vanrell, DH. Milone, SA. Utsumi, JR. Galli, HL. Rufiner and LL. Giovanini (2016) A real-time algorithm for acoustic monitoring of ingestive behavior of grazing cattle. *Computers and Electronics in Agriculture,* 127: 64–75.

Clapham, WM., JM. Fedders, K. Beeman and LPS. Neel (2011) Acoustic monitoring system to quantify ingestive behavior of free-grazing cattle. *Computers and Electronics in Agriculture,* 76: 96–104.

Flores, ER., FD. Provenza and DF. Balph (1989) The effect of experience on the foraging skill of lambs: Importance of plant form. *Applied Animal Behaviour Science,* 23: 285–291.

Galli, JR., CA. Cangiano, DH. Milone and EA. Laca (2011) Acoustic monitoring of short-term ingestive behavior and intake in grazing sheep. *Livestock Science,* 140: 32–41.

Gozález-Pech, PG., JFJ. Torres-Acosta and CA. Sandoval-Castro (2014) Adapting a bite coding grid for small ruminants browsing a deciduous tropical forest. *Tropical and Subtropical Agroecosystems,* 17: 63–70.

Hirata, M., T. Tanikawa and M. Tobisa (2011) Quantifying short-term foraging behavior of cattle grazing a tall grass: A preliminary study with maize. *Livestock Science,* 138: 220–228.

Laca, EA. and MF. Wallis De Vries (2000) Acoustic measurement of intake and grazing behaviour of cattle. *Grass and Forage Science,* 55: 97–104.

Laca, EA., ED. Ungar and MW. Demment (1994) Mechanisms of handling time and intake rate of a large mammalian grazer. *Applied Animal Behaviour Science,* 39: 3–19.

Laca, EA., ED. Ungar, NG. Seligman, MR. Ramey and MW. Demment (1992a) An integrated methodology for studying short-term grazing behaviour of cattle. *Grass and Forage Science,* 47: 81–90.

Laca, EA., ED. Ungar, NG. Seligman and MW. Demment (1992b) Effects of sward height and bulk density on bite dimensions of cattle grazing homogeneous swards. *Grass and Forage Science,* 47: 91–102.

Milone, DH., JR. Galli, CA. Cangiano, HL. Rufiner and EA. Laca (2012) Automatic recognition of ingestive sounds of cattle based on hidden Markov models. *Computers and Electronics in Agriculture,* 87: 51–55.

Milone, DH., HL. Rufiner, JR. Galli, EA. Laca and CA. Cangiano (2009) Computational method for segmentation and classification of ingestive sounds in sheep. *Computers and Electronics in Agriculture,* 65: 228–237.

Nakano, M. and S. Ogura (2017) Effect of plant-species richness on microbial composition and rumen function. *Journal of Integrated Field Science,* 14: 33-37 .

Novellie, PA. (1978) Comparison of the foraging strategies of blesbok and springbok on the Transvaal highland. *South African Journal of Wildlife Research,* 8: 137–144.

Ogura, S., H. Mizuno, S. Takamizawa, M. Yayota and K. Kawamura (2017) Nutritional characteristics of forbs and tree leaves and their contribution to animal production in species-rich vegetation. *Journal of Integrated Field Science,* 14: 23-27.

Penning, PD. and SM. Rutter (2004) Ingestive behaviour. In: Herbage Intake Handbook (2nd Edition), P.D. Penning (Ed), The British Grassland Society, Reading, pp.151–175.

Rutter, SM., RA. Champion and PD. Penning (1997) An automatic system to record foraging behaviour in free-ranging ruminants. *Applied Animal Behaviour Science*, 54: 185–195.

Ruyle, GB. and DD. Dwyer (1985) Feeding station of sheep as an indicator of diminished forage supply. *Journal of Animal Science,* 61: 349–353.

Senft, RL., MB. Coughenour, DW. Bailey, LR. Rittenhouse, OE. Sala and DM. Swift (1987) Large herbivore foraging and ecological hierarchies. *BioScience*, 37: 789–795+798-799.

Tamiya, S., K. Doi, N. Nakajima, S. Ogura and M. Yayota (2016) Effect of plant diversity on ruminal degradability of goats grazing in a semi-natural pasture. *Journal of Integrated Field Science*, 14: 107.

Ungar, ED. (1996) Ingestive behaviour. In: The Ecology and Management of Grazing Systems, J. Hodgson J and Illius AW (Eds), CAB International, Wallingford, pp.185–218.

Wang, L., DL. Wang, Z. He, G. Liu and KC. Hodgkinson (2010) Mechanisms linking plant species richness to foraging of large herbivore. *Journal of Applied Ecology,* 47: 868–875.

Yayota, M., A. Kato, M. Ishida and S. Ohtani (2015) Ingestive behavior and short-term intake rate of cattle grazing on tall grasses. *Livestock Science,* 180: 113–120.

Yoshihara, Y., H. Mizuno, S. Ogura and S. Sato (2013) Increasing the number of plant species in a pasture improves the mineral balance of grazing beef cattle. *Animal Feed Science and Technology,* 179: 138–143.

Contribution of Rearing at Pasture on Improvement of Animal Welfare in Fattening Pigs

Akitsu TOZAWA[1] and Shusuke SATO[2]

[1]Graduate School of Agricultural Science, Tohoku University, Osaki, Miyagi, 989-6711, Japan
[2]Faculty of Life and Environmental Sciences, Teikyo University of Science, Adachi-ku, Tokyo, 120-0045, Japan

Corresponding Author: Akitsu Tozawa, akitsu.tozawa.c2@tohoku.ac.jp

Introduction

Maintaining the health of animals is an important part of livestock management. Ensuring good health of livestock has several benefits. Not only does it help prevent economic losses for farmers (e.g. the cost of treating diseased animals), it also protects human health by reducing the chances of zoonotic infections and providing a better guarantee of food. As animal health and animal welfare are closely related, World Organisation for Animal Health (OIE), which supervises animal health worldwide, has emerged as the leading international organization for animal welfare (OIE, 2014). OIE has six primary objectives that include ensuring food safety and improving animal welfare. This has led to a better guarantee of animal products, as ensuring animal welfare during livestock management has recently become a global standard for farmers.

Rearing of pigs at pastures is expected to improve animal welfare (FAWC, 1996). However, there is a possibility of deterioration in physical health given by the severity of outdoor environment. To understand the contribution of rearing at pasture on improvement of animal welfare, we compared the behavior, physical health, and productivity of fattening pigs reared in an outdoor pasturing system compared to an indoor intensive system.

Behavior

To compare the behavior of pigs between rearing systems, two treatments were prepared, reproduction of rearing systems as an outdoor pasturing system (OP) and an indoor intensive system (IS) (Tozawa *et al.*, 2016b). OP had an open-air roofed pen (1.2 m^2/head) and a pasture (200 m^2). IS had a pen, similar to OP, but no associated pasture. OP and IS treatments were in the same field to ensure similar air condition and temperature. Four castrated males (LW breed) were offered to each treatment. They were introduced to the treatment at 87 days of age and reared until they were 193 days old. When the pigs were 124 days old, the rearing site for OP was changed to an adjacent pasture of similar size due to depletion of grass in the previous pasture. Feed and water were available *ad libitum* and the same feed concentrate was provided in both treatments. Maintenance behaviors and behavior as events (Table 1) in the pigs were observed at 21 weeks of age.

Table 2 shows the time budget of maintenance behaviors in pigs. The time budget for foraging, which included the feeding, grass eating, and soil eating time was 19.9 ± 3.5% (mean ± SD) in OP and 12.7 ± 1.0% in IS (P = 0.03). The time budget for the feed concentrate was the same for OP and IS pigs, whereas OP pigs had a higher grass eating and soil eating budget. This resulted in a significantly higher foraging time for OP pigs than the IS pigs. In addition, OP pigs had a 3.7-fold higher exploration time compared with IS pigs (P = 0.03). Finally, this higher expression of foraging and exploring in OP pigs was associated with increased activity. Pig is an inquisitive animal that shows a highly motivated exploring behavior (Wood-Gush *et al.*, 1990). If pigs are reared in an unstimulating environment, they tend to be apathetic (Ruiterkamp, 1987) and undergo chronic stress (Beattie *et al.*, 2000). Barren environments also appear to have pronounced effects such as impairment of cognitive function and increased fearfulness in pigs (Beattie *et al.*, 2000). The outdoor pasturing system could have provided a stimulating environment, because of which, the pigs showed a high exploration and active behavior, leading to their improved welfare.

Table 1. *Ethogram of maintenance behavior and behavior as events.*

Behavior	
Maintenance behavior	
Foraging	Total time of feeding, grass eating and soil eating
Feeding	Gathering, chewing and swallowing concentrate
Grass eating	Chewing and swallowing fresh grass in pasture
Soil eating	Chewing and swallowing soil in pasture
Exploring	Sniffing surrounding environments, rooting, chewing rocks
Activity	The time except resting
Behavior as events	
Agonistic behavior	Head butting or aggressive biting at other pen mates
Affilative behavior	Smelling, licking other pen mates
Chewing	Chewing action with if the objects (grass, soil, or food) were seen in the pigs' mouth
Rooting	Back-and-forward movement of the snout over soil, but not pen mates
Disturbed behavior	Chewing or rooting pen mates, chewing or biting facilities, sham-chewing
Play	Bouts of chasing, fight with others, play at a puddle

Modified from Tozawa *et al*. (2016b)

Table 2. *Time budgets of maintenance behavior of pigs.*

	Rearing system[1]		
	OP	IS	*P*-value[2]
Foraging	19.9 ± 3.5	12.7 ± 1.0	0.03
Feeding	12.7 ± 2.7	12.7 ± 1.0	0.69
Grass eating	5.2 ± 1.6	0	0.01
Soil eating	2.1 ± 0.6	0	0.01
Exploring	12.2 ± 1.0	3.3 ± 0.8	0.03
Activity	45.2 ± 6.7	25.7 ± 4.0	0.03

Modified based on Tozawa *et al*. (2016b)
Value are expressed as mean ±S.D.% in the observation period
[1]OP: Outdoor pasturing system, IS: Indoor intensive system
[2]Mann-Whitney U test

Table 3. *Frequencies of behavioral as events of pigs.*

	Rearing system[1]		
	OP	IS	*P*-value[2]
Agonistic behavior	1.6 ± 0.6	1.7 ± 0.9	0.77
Affilative behavior	0.7 ± 0.8	1.1 ± 0.4	0.45
Rooting	13.9 ± 6.0	0	0.01
Chewing	19.4 ± 3.6	0.3 ± 0.3	0.02
Disturbed behavior	0.7 ± 0.8	8.3 ± 5.9	0.02
Play	0.7 ± 1.0	0	0.05

Modified based on Tozawa *et al*. (2016b)
Value are expressed as mean ±S.D. (Expression times / h)
[1]OP: Outdoor pasturing system, IS: Indoor intensive system
[2]Mann-Whitney U test

Table 3 shows the frequencies of behavior as events of pigs. Agonistic behavior and affiliative behavior had any significant difference between the treatments. These social behaviors were exhibited equally, regardless of the rearing system. The expression of chewing grass, soil, or concentrate was 19.4 ± 3.6 times/h (mean ± SD) in OP pigs and 0.3 ± 0.3 times/h in IS pigs (P = 0.02). Rooting soil behavior using the nose was expressed 13.9 ± 6.0 times/h in OP pigs but had no expression in IS pigs (P = 0.01). When pigs do not get access to substrates for expressing chewing, rooting, and snouting, these exploratory behaviors are often redirected towards pen-mates and facilities (Peterson *et al.,* 1995; Beattie *et al.,* 2000). Such redirected behavior is one of the most severe problems for pigs reared in an intensive system, leading to body wounds (e.g., tail biting) or destruction of facilities. IS pigs expressed disturbed behavior 11.9 times more than the OP pigs (P = 0.02). In addition, IS did not express any playing behavior whereas the OP pigs expressed it 0.7 ± 1.0 times/h (P = 0.05). Disturbed behavior, including redirected behavior, is indicative of mental conflict in pigs that could not express enough of their natural behavior. In contrast, playing behavior accompanies a positive emotion such as enjoyment (Olsen *et al.,* 2002). Pasture rearing not only results in a natural and active behavior of pigs, but they also express less behavior-associated stress and more behavior accompanies positive emotion, leading to improved overall welfare.

Physical health

Physical health is an important component of animal welfare. Lesions of lung disease and wounds on the body were examined for estimating physical health.

One of the health problems in intensive system of fattening pigs is the lung disease. In particular, Mycoplasmal pneumonia of swine (MPS) is quite common and its infection rate is extremely high worldwide. Improving the air condition suppresses the damage of lung by MPS (Yagihashi *et al.,* 1993). Therefore, we hypothesized that the outdoor conditions of pasture might have an effect on it. The number of affected pigs and the percentage invasiveness of MPS lung lesions were observed (Tozawa *et al.,* 2016a) using an established scoring system (Goodwin and Whittlestone, 1973). The observed pigs were introduced from the same farm, but reared in different systems, 2 outdoor pasturing systems and an indoor intensive system, with 14 pigs per system. The number of affected pigs (P = 0.06) and the percentage invasiveness of MPS lung lesions (P = 0.51) did not show any difference between the rearing systems. The main difference between the rearing systems was the air condition, which depended on the availability of an outdoor environment. As no difference was observed between the rearing systems, these traits might instead depend on the disease management strategy at the introducing farm.

Wounds on the body for the same pigs that were reared at OP and IS for behavioral observations, were scored using Welfare Quality® assessment protocol (Welfare Quality® Consortium, 2009) before shipping (Tozawa et al., 2016b). The score for OP pigs was 6.3 ± 4.6 points (mean ± SD) whereas that of IS pigs was 47.5 ± 22.7 points (P = 0.03). The occurrence of body wounds increased with an increased expression of aggression or disturbed behavior from other pigs. The OP pigs expressed less disturbed behavior and behaved naturally. As a result, they had less behavior-related stress. Satisfactory expression of natural behavior could be the cause of lower wound score in OP pigs. Severe body wounds increase the risk of infection, and result in reduced weight gain because of pain-associated appetite loss (Schrøder-Petersen and Simonsen, 2001). Fewer wounds in OP pigs would reduce the chances of infection and keep them healthy, in addition to increasing the productivity.

Productivity

Body weight of pigs and skatole (one of the meat constituents that affects its smell) were measured for estimating productivity.

Body weight was measured for the same pigs that were reared at OP and IS for behavioral observations. When they were introduced into the treatment, OP pigs weighed 32.9 ± 1.1 kg (mean ± SD) and IS pigs weighed 36.0 ± 1.1 kg. OP pigs were significantly lighter than the IS pigs (P = 0.04). However, OP pigs grew to 119.9 ± 4.6 kg and IS pigs grew to 119.3 ± 11.5 kg before shipping, and there was no significant difference between the two groups (P = 0.57). The average daily gain (ADG) was calculated during at the prior period (from introduction to treatments until reaching around 70 kg of weight) and the latter period (from around 70 kg to 100 kg of weight) (Tozawa *et* *al.*, 2016b). During the prior period, the ADG for OP pigs was 0.68 ± 0.18 kg/d and that of IS pigs was 0.76 ± 0.23 kg/d, with no significant difference (P = 0.70). In contrast, the ADG in OP (0.91 ± 0.09 kg/d) tended to be higher than in IS (0.67 ± 0.15 kg/d) during the latter period (P = 0.06). In other words, pigs reared in pasture showed a greater increase in body weight during the latter fattening period. These results show a difference between pigs reared in pasture and pigs reared in an indoor intensive system with respect to their growth period. The concern that OP pigs might lose weight by virtue of being more active than IS pigs was found to be untrue, as the body weights did not differ at the time of shipping.

The meat of pasture-reared pigs is considered less smelly than the meat sold in general, but there is not enough scientific evidence in this regard. A strong smell might affect the consumers' interests in buying the meat. Skatole is an aromatic compound that absorbed from the gastrointestinal tract, skin or lung (Hansen *et al.*, 1994). Skatole in the backfat of 9 pigs (5 reared in pasture and 4 reared in indoor intensive system) was analyzed. The proportion of animals with less than 0.01 ppm concentration of skatole in backfat was 4 out of 5 for pasture-reared pigs and 2 out of 4 for indoor intensive system pigs (Tozawa *et al.*, 2016a). A study on pigs raised in commercial farms reported that only 4 of 128 pigs had a fat skatole concentration lower than 0.01 ppm (Nishioka *et al.*, 2011). In comparison, the proportion of pasture-reared pigs with a skatole concentration in backfat lower than 0.01 ppm was remarkably large. According to a report based on sensory tests, the range of values for which people can smell skatole was 0.008-0.060 μg/g (Annor-Frempong *et al.*, 1997). Smell perception can vary among individuals as it is a sensitive ability. Even a concentration of skatole as low as 0.01 ppm could affect the consumers' preferences. The effect of pasture rearing on the flavor of meat could be considered as small, as there was a low concentration of skatole in the backfat of pasture-reared pigs.

Conclusion

Comparing between pigs reared in an outdoor pasturing system and an indoor intensive system, pigs reared at the pasture behaved more naturally and acted with positive emotion. These results show that satisfactory expression of natural behavior leads to a decrease in psychological conflict. Furthermore, pigs at the pasture maintained a better physical health due to fewer wounds on the body. Rearing pigs at the pasture improved the welfare not only improving by leading mental health, but also maintained physical health. In addition, there is a possibility of added benefit as the meat might not have a strong smell.

Acknowledgment

A part of the project was supported by the Ito Foundation. We gratefully acknowledge the support from the farm staff at Field Science Center and the staff at Laboratory of Land Ecology and Animal Welfare, Graduate School of Agricultural Science, Tohoku University, in setting up the experiments.

References

Annor-Frempong, I.E., G.R. Nute, F.W. Whittington and J.D. Wood (1997) The problem of taint in pork: 1. Detection thresholds and odour profiles of androstenone and skatole in a model system. Meat Sci, 46: 45–55.

Beattie, V.E., N.E. O'Connell, D.J. Kilpatrick and B.W. Moss (2000) Influence of environmental enrichment on welfare-related behavioural and physiological parameters in growing pigs. Anim Sci, 70: 443–450.

FAWC (1996) Report on the welfare of pigs kept outdoors. Farm Animal Welfare Council, Tolworth, UK.

Goodwin, R.F. and P. Whittlestone (1973) Enzootic pneumonia of pigs: immunization attempts inoculating *Mycoplasma suipneumoniae* antigen by various routes and with different adjuvants. Br Vet J, 129: 456–464.

Hansen, L.L., A.E. Larsen, B.B. Jensen, J. Hansen-Møller and P. Bartongade (1994) Influence of stocking rate and faeces deposition in the pen at different temperatures on skatole concentration (boar taint) in subcutaneous fat. Anim Prod, 59: 99–110.

Nishioka, T., Y. Ishizuka, Y. Inno and M. Irie (2011) Skatole concentration in porcine fat and its influence on sensory evaluation. Nihon Chikusan Gakkaiho, 82: 147–153. (in Japanese)

OIE (2014) OIE's achievements in animal welfare. World Organisation for Animal Health. http://www.oie.int/en/animal-welfare/animal-welfare-key-themes/ (date of access: Nov. 10, 2016)

Olsen, A.W., H.B. Simonsen and L. Dybkjaer (2002) Effect of access to roughage and shelter on selected behavioural indicators of welfare in pigs housed in a complex environment. Anim Welf, 11: 75–87.

Peterson, V., H.B. Simonsen and L.G. Lawson (1995) The effect of environmental stimulation on the development of behavior in pigs. Appl Anim Behav Sci, 45: 215–224.

Ruiterkamp, W.A. (1987) The behaviour of growing pigs in relation to housing systems. Netherlands J Agric Sci, 35: 67–70.

Schrøder-Petersen, D.L. and H.B. Simonsen (2001) Tail biting in pigs. Vet J, 162: 196–210.

Tozawa, A., T. Takahashi and S. Sato (2016a) Effects of pasturing system on behaviour, lung disease, enteric bacteria and back fat skatole in fattening pigs. Tohoku J Anim Sci Technol, 66: 23–32. (in Japanese)

Tozawa, A., S. Tanaka and S. Sato (2016b) The effects of components of grazing system on welfare of fattening pigs. Asian Australas J Anim Sci, 29: 428–435.

Welfare Quality® Consortium (2009) Welfare Quality® assessment protocol for pigs (sows and piglets, growing and finishing pigs). Welfare Quality® Consortium, Lelystad, Netherlands.

Yagihashi, T., S. Kazama and M. Tajima (1993) Seroepidemiology of mycoplasmal pneumonia of swine in Japan as surveyed by enzyme-linked immunosorbent assay. Vet Microbiol, 34: 155–166.

Wood-Gush, D.G.M., K. Vestergaard and V. Petersen (1990) The significance of motivation and environment in the development of exploration in pigs. Biol Behav, 15: 39–52.

JIFS, 14 : 55－59 (2017)

Effects of Environmental Enrichment on Welfare of Cattle

Siyu CHEN[1] and Shin-ichiro OGURA[2]

[1]State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, 100193, China
[2]Laboratory of Land Ecology, Graduate School of Agricultural Science, Tohoku University, Miyagi 989-6711, Japan

Corresponding Author: Siyu. CHEN, jihebuluo2015@163.com

Keywords:animal welfare, cattle, environmental enrichment

Abstract

In recent years, keeping animals indoors has become a popular rearing system in many countries where there is a high population density and lack of land to graze animals. However, this frustrates animals by depriving them of opportunities for many behaviors. To solve these problems, environmental enrichment was introduced to animal welfare science. It is thought to be an alternative means to contribute to animal welfare. It focuses on the biological functions of the animals and helps them to cope with stressors in their surroundings, prevent frustration, and express more normal behaviors. In this review, several elements of environmental enrichment and how they affect animal welfare are discussed. First, we discuss natural suckling and group rearing, both of which allow animals to perform social behaviors with other individuals. Second, we summarize behavior-stimulating tools such as brushing, scratching/rubbing arch devices, and bedding materials. Finally, space allowance as physical enrichment is discussed. Now that animal welfare science focuses on how to improve animal welfare and prevent stress, rather than reducing stress when animals have already suffered, therefore more attentions should be paid to studies on environmental enrichment.

Introduction

In recent years, keeping animals indoors has become a leading rearing system in many countries where there is a high population density and lack of land to graze animals. Rearing animals indoors provides many advantages, such as protecting animals from extreme weather conditions and parasite infections and reducing environmental pollution. However, it also frustrates animals, resulting in a change in behavioral and physiological indicators. For example, disturbed behavior, such as repeated tail-biting (Schrøder-Petersen and Simonsen, 2001) in pigs, and severe feather-pecking and vacuum nest-building in chickens (Jensen, 1993), occurs during unnatural life conditions. As to physiology, it has been reported that cortisol concentration is increased by social disruption (Adeyemo and Heath, 1982), restraint (Lefebvre et al., 1990), and transport (Palme et al., 2000). Cattle with high levels of serum cortisol spend less time ruminating and vocalize more than those with low levels of cortisol (Bristow and Holmes, 2007).

Until recently, most welfare assessments were conducted when animals were already under stressful conditions. However, studies of how to improve welfare and keep animals in a comfortable condition and prevent stress should be paid more attentions than studies on how to reduce stress after it has occurred. Duncan and Olsson (2001) argued that freedom from the state of suffering is assured by providing for environmental requirements, while the establishment of pleasurable states requires environmental enrichment. Environmental enrichment is widely used to help animals cope with stress in indoor rearing.

On the other hand, environmental enrichment is an alternative way to contribute to animal welfare. It focuses on the animals' biological functions, helps animals to cope with stressors in their surroundings, prevents frustration, and allows animals to express more normal behaviors (Newberry, 1995). Bloomsmith et al. (1991) stated that environmental enrichment can be divided into five categories: social, occupational, physical, sensory, and nutritional. Therefore, environmental enrichment can improve animal welfare from many angles. For example, opportunities to engage in complex social interactions may fill the calves' need for a companion and thus reduce stress (De et al., 2012). This increases the fitness of the animals' biological functioning, including lifetime reproductive success.

In this review, we focus on several elements of environmental enrichment and how they play roles in cattle welfare.

Elements of environmental enrichment

Rearing systems

It is well known that in intensive production systems, calves are separated from their dams immediately or within a few hours after birth. Under these circumstances, calves may be deprived of many natural behaviors and biological functions. Social behaviors, such as contact with peers, are present from the first week of life (Wood-Gush et al., 1984).

Artificial suckling might suppress the normal suckling behavior of natural lives, contributing to the onset of stereotypies such as tongue playing in Japanese Black cattle (Sato et al., 1994). In comparison, natural suckling systems, in which calves are nursed by their dams and have social contact with other calves and cows, have a positive effect on daily weight gain and vitality in calves (Krohn, 2001). Further, calves reared with their dams struggle less when restrained for blood sampling compared with those housed singly (Duve et al., 2012), and contact with the mother has a long-term effect on behavior (Wagner et al., 2012). Natural suckling also has effects on physiology. An instantaneous increase in serum oxytocin concentrations has been reported in calves during natural suckling (Lupoli et al., 2001). In a previous study, we showed that the basal serum oxytocin concentration was higher in calves of one-month-old under the natural suckling system than under the bucket-suckling system. We concluded that natural suckling might contribute to an increase in investigative behavior via the increase of serum oxytocin concentration (Chen et al., 2015).

Group rearing also benefits the animals. Duve and Jensen (2012) reported that calves reared in groups performed more social behaviors than calves housed individually, with limited social contact, from the age of three weeks. Further, group-housed calves spent less time alone and had a higher social rank when introduced into a new group than individually housed calves (Broom and Leaver 1978).

Social interaction with dams and peers is important to calves. Wood-Gush et al. (1984) reported a higher frequency of interactions of calves and their dams until four weeks of age (0.72/30 min) than with other cows (0.18/30 min) or calves (0.68/30 min). As a consequence, natural and group rearing fulfill the need for social behaviors in cattle, and thus contribute to animal welfare.

Behavior-stimulating tools

All domestic animals are strongly motivated to explore and investigate when they face a new environment (Broom and Fraser, 2007). However, barren indoor housing deprives calves of this behavior, and so behavior-stimulating tools have been developed to improve this situation.

It has been suggested that social grooming in cows is an important behavior pattern with functional significance for the formation and maintenance of social bonds; it is regarded as a reliable indicator of friendship (Sato et al., 1991; Boissy et al., 2007). The pattern of this behavior changes with indoor housing, but brushing can fulfill this need in cows. Brushing is thought to act by a similar mechanism to that of maternal grooming, in that they both have a cleaning effect on animals (Kohari et al., 2009; Schukken and Young, 2009). Further, it has been reported that brushing is widely used by farmers in the livestock industry for various purposes, including to increase milk yield (Schukken and Young, 2009), and an effect of building a physiological bond between the stockperson and animal is expected. Ninomiya and Sato (2009) reported that brushing promoted self-grooming behavior and improved welfare in Japanese Black and Japanese Shorthorn calves. Meanwhile, brushing connects to physiological indicators. In a previous study, we found that manual brushing increased serum oxytocin concentrations in cattle (Chen et al., 2014). Since oxytocin is one of the positive indicators of livestock welfare (Broom and Fraser, 2007), this suggests a positive effect of brushing on cattle. Taking the above into consideration, brushing may be an alternative mean to provide social grooming behavior for cows housed indoors.

In outdoor rearing, cows can scratch themselves and rub on trees in the pasture. This behavior is inhibited when they are reared in tie-stalls or narrow cowsheds. An arch-shaped device for scratching and rubbing, which allows cattle to scratch their dorsal and head areas, has been designed to let animals perform this behavior (Simonsen, 1979). In an applied study, it was reported that cows used these arches for longer durations and higher frequencies than other devices (Wilson et al., 2002). In addition, studies have investigated designs to allow cattle to express exploration behavior. It has been reported that a soil floor stimulated explorative behaviors and improved the health and welfare of Japanese Black steers (Ariga et al., 2015). Thus, it was concluded that a soil floor provides novelty and enrichment for cattle, compared to a concrete floor.

Space allowance

Physical enrichment is also an important enrichment for cows (Bloomsmith et al., 1991). Animals will be stressed if adequate space is not provided.

In many countries, dairy cows are kept throughout the year in tie-stalls, or narrow cowsheds, which results in a lack of exercise and contact with other cows. It has been reported that calves that had greater space allowance performed higher levels of play behavior (Jensen et al., 2015). Similarly, in adult cows, when space allowance increased, agonistic behavior decreased (Kondo et al., 1989).

However, space allowance is not just about providing constant visual and physical contact between conspecifics, but also providing an opportunity to separate from other individuals on some occasions. For example, dairy cows seek isolation from calve when ill, suggesting that providing a cow with a secluded area may provide a physical enrichment benefit to it (Proudfoot et al., 2014). Even in natural life, animals need individual space. Most cows separate themselves from others when calving, and select dry and high-altitude places with tree cover and branches overhead or a shelter (Lidfors et al., 1994).

Animals need contact with others to perform social behaviors, but also private space to comfort themselves at times. These two needs should be considered when designing housing and husbandry systems.

Conclusions

Recently, studies on positive emotional states, rather than negative states, in animals have started to receive greater interest (Boissy et al., 2007; Mellor 2012; Proctor and Carder, 2014). Required conditions for animal welfare have been proposed as the five freedoms: freedom from hunger and thirst; freedom from discomfort; freedom from pain, injury, or disease; freedom to express normal behavior; and freedom from fear and distress (FAWC, 1992). Thus, environmental enrichment as an element of improving animal welfare has attracted interest from many scientists.

In the present review, we focused on several elements of environmental enrichment that may help cattle to cope with stressors in their surroundings, prevent frustration, and perform more normal behaviors. These methods are based on meeting the biological needs of the animals. As described above, these are natural and group rearing, behavior-stimulating tools to allow for some natural behaviors, and space allowance to perform normal behaviors. In this case, we suggest that studies on environmental enrichment should be paid further attention.

References

Adeyemo, O. and E. Heath (1982) Social behaviour and adrenal cortical activity in heifers. Appl. Anim. Ethol. 8: 99–108.

Ariga, S., S. Tanaka, T. Chiba, K. Shibuya, I. Kajiwara I, S. Chen and S. Sato (2015) Long-term exercise on soil floor improves the health and welfare of Japanese Black steers. Proceedings of the 49th of the International Society for Applied Ethology, p.140.

Bloomsmith, M.A., L.Y. Brent and S.J. Schapiro (1991) Guidelines for developing and managing an environmental enrichment program for nonhuman primates. Lab. Anim. Sci, 41: 372–377.

Boissy, A., G. Manteuffel, M.B. Jensen, R.O. Moe, B. Spruijt, L.J. Keeling, C. Winckler, B. Forkman, I. Dimitrov, J. Langbein, M. Bakken, I. Veissier and A. Aubert (2007) Assessment of positive emotions in animals to improve their welfare. Physiol. Behav, 92: 375–397.

Bristow, D.J. and D.S. Holmes (2007) Cortisol levels and anxiety-related behaviors in cattle. Physiol. Behav, 90: 626–628.

Broom, D.M. and A.F. Fraser (2007) Welfare Assessment in Direct Measures of Good Welfare. In Domestic animal behaviour and welfare, 4th edition, Broom, D.M. and A.F. Fraser (Eds.), p.65, Cambridge University Press, London.

Broom, D.M. and J.D. Leaver (1978) Effects of group-rearing or partial isolation on later social behaviour of calves. Anim. Behav. 26: 1255–1263.

Chen, S., S. Tanaka, S. Roh and S. Sato (2014) Factors affecting oxytocin concentration and relationship between oxytocin concentration and affiliative behavior. Proceedings of the 48th of the International Society for Applied Ethology, p.137.

Chen, S., S. Tanaka, S. Ogura, S. Roh and S. Sato (2015) Effect of suckling systems on serum oxytocin and cortisol concentrations and behavior to a novel object in beef calves. Asian-Aus. J Anim. Sci, 28: 1662–1668.

De P.V.A., A.M. de Passillé and D.M. Weary (2012) Effects of the early social environment on behavioral responses of dairy calves to novel events. J. Dairy Sci, 95: 5149–5155.

Duncan, I.J.H. and I.A.S. Olsson (2001) Environmental enrichment: from flawed concept to pseudoscience. Proceedings of 35th International Society for Applied Ethology, p.73.

Duve, L.R., D.M. Weary, U. Halekoh and M.B. Jensen (2012) The effects of social contact and milk allowance on responses to handling, play, and social behavior in young dairy calves. J. Dairy Sci, 95: 6571–6581.

Duve, L.R. and M.B. Jensen (2012) Social behavior of young dairy calves housed with limited or full social contact with a peer. J. Dairy Sci, 95: 5936–5945.

Farm Animal Welfare Council (FAWC) (1992) Five Freedoms. London, UK.

Jensen, P (1993) Nest building in domestic sows: the role of external stimuli. Anim. Behav, 45: 351–358.

Jensen, M.B., L.R. Duve and D.M. Weary (2015). Pair housing and enhanced milk allowance increase play behavior and improve performance in dairy calves. J. Dairy Sci, 98: 2568–2575.

Kohari, D., S. Sato and Y. Nakai (2009) Does the maternal grooming of cattle clean bacteria from the coat of calves? Behav. Processes, 80: 202–204.

Kondo, S., J. Sekine and M. Okubo (1989) The effect of group size and space allowance on the agonistic and spacing behavior of cattle. Appl. Anim. Behav. Sci, 24:127–135.

Krohn, C.C (2001) Effects of different suckling systems on milk production, udder health, reproduction, calf growth and some behavioural aspects in high producing dairy cows — a review. Appl. Anim. Behav. Sci, 72: 271–280.

Lefebvre, D.M., G.F. Gallo and E. Block (1990) The effect of assistance at calving, injections of recombinant bovine somatotropin and jugular catheterization on serum cortisol and its influence on the GnRH-induced LH response in postpartum dairy cows. Can. J. Anim. Sci, 70: 723–726.

Lidfors., L.M., D. Moran, J. Jung, P. Jensen and H. Castren (1994) Behaviour at calving and choice of calving place in cattle kept in different environments. Appl. Anim. Behav. Sci, 42: 11–28.

Lupoli, B., B.Johansson, K.Uvnäs-Moberg and K. Svennersten-Sjaunja (2001) Effect of suckling on the release of oxytocin, prolactin, cortisol, gastrin, cholecystokinin, somatostatin and insulin in dairy cows and their calves. J. Dairy Res, 68: 175–187.

Mellor, D.J (2012) Animal emotions, behaviour and the promotion of positive welfare states. New Zealand Vet. J, 60: 1–8.

Newberry, R.C (1995) Environmental enrichment: increasing the biological relevance of captive environments. Appl. Anim. Behav. Sci, 44: 229–243.

Ninomiya, S. and S. Sato (2009) Effects of ‘Five freedoms’ environmental enrichment on the welfare of calves reared indoors. Anim. Sci. J, 80: 347–351.

Palme, R., C. Robia, W. Baumgartner and E. Möstl (2000) Transport stress in cattle as reflected by an increase in faecal cortisol metabolite concentrations. Vet. Rec, 146: 108–109.

Proudfoot, K.L., M.B. Jensen, D.M. Weary and M.A.G. von Keyserlingk (2014) Dairy cows seek isolation at calving and when ill. J. Dairy Sci, 97: 2731–2739.

Proctor, H. S. and G. Carder (2014) Can ear postures reliably measure the positive emotional state of cows? Appl. Anim. Behav. Sci, 161: 20–27.

Sato, S., S. Sako and A. Maeda (1991) Social licking patterns in cattle (*Bos taurus*): influence of environmental and social factors. Appl. Anim. Behav. Sci, 32: 3–12.

Sato, S., R. Nagamine and T. Kubo (1994) Tongue-playing in tethered Japanese Black cattle: diurnal patterns, analysis of variance and behaviour sequences. Appl. Anim. Behav. Sci, 39: 39–47.

Schrøder-Petersen, D.L. and H.B. Simonsen (2001) Tail Biting in Pigs. Vet. J, 162: 196–210.

Schukken, Y. H. and C.D. Young (2009) Field study on milk production and mastitis effect of the

Delaval Swinging Cow Brush. Delaval Swinging Cow Brush Study Final Report, August 5. Delaval, Tumba, Sweden.

Simonsen, H. B (1979) Grooming behaviour of domestic cattle. Nordisk Veterinaermedicin, 31: 1–5.

Wagner, K., K. Barth, R. Palme, A. Futschik and S. Waiblinger (2012) Integration into the dairy cow herd: Long-term effects of mother contact during rearing the first twelve weeks of life. Appl. Anim. Behav. Sci, 141:117–129.

Wilson, S.C., F.M. Mitlöhner, J. Morrow-Tesch, J.W Dailey and J.J.Mcglone (2002) An assessment of several potential enrichment devices for feedlot cattle. Appl. Anim. Behav. Sci, 76: 259–265.

Wood-Gush, D.G.M., K. Hunt, K. Carson and S.G.C. Dennison (1984) The early behaviour of suckler calves in the field [social behaviour, creche behaviour, behavioural sequences]. Biol. Behav, 9: 295–306.

Monitoring Spatial Heterogeneity of Pasture within Paddock Scale using a Small Unmanned Aerial Vehicle (sUAV)

Kensuke KAWAMURA[1], Jihyun LIM[2], Yuzo KUROKAWA[3], Taketo OBITSU[4], Masato YAYOTA[5] and Shin-ichiro OGURA[6]

[1]Japan International Research Center for Agricultural Sciences (JIRCAS), Japan
[2]Global Career Design Center, Hiroshima University, Japan
[3]Setouchi Field Science Center, Graduate School of Biosphere Science, Hiroshima University, Japan
[4]Graduate School of Biosphere Science, Hiroshima University, Japan
[5]Faculty of Applied Biological Science, Gifu University, Japan
[6]Graduate School of Agricultural Science, Tohoku University, Japan

***Correspondence: Kensuke KAWAMURA**
Tel: +81-29-838-6614 Fax: +81-29-838-6342
e-mail: kamuken@affrc.go.jp

Keywords: drone, forage nutrient, grazing management, spatial heterogeneity

Abstract

Site-specific management strategies in a grazing ecosystem increase management efficiency. Due to the complex interrelationship among the soil-plant-animal-environment in a grazing ecosystem, site-specific grazing management requires a high measurement density to reflect their spatial patterns within a paddock rather than an inter-paddock scale. Recently, small unmanned aerial vehicles (sUAV) have been introduced into agricultural research. Their images offer a potential alternative for pasture monitoring given by their low cost of operation, high spatial and temporal resolution, and their high flexibility in image acquisition programming. In this study, we reviewed current developments of sUAVs and photogrammetric algorithms, and we highlight the applications for pasture managements using results obtained mainly at Hiroshima University farm, which included; (1) seasonal changes in a number of species and the nutrient status of plants, (2) the spatial distribution of herbage biomass from sUAVs, and (3) the spatial distribution of GPS collars attached to cows.

1. Introduction

Site-specific grazing management by assessing plant productivity and species richness in a grazing ecosystem is regarded as a central task for the efficient management and conservation of the ecosystem. Species richness generally promotes ecosystem productivity, and the species richness-productivity relationship has been of interest in grassland ecosystems (Wang *et al.*, 2016). Moreover, a species-rich ecosystem creates rich variations of nutrient composition for large herbivores to feed on (Mizuno *et al.* 2014; Takamizawa *et al.* 2016b). The species rich conditions may also affect their diet selection and foraging behavior (Ogura *et al.* 2011).

Meanwhile, grazing by large herbivores also affects herbage productivity and plant species richness in grassland ecosystems. The grazing activities influence the availability of essential resources, such as light and soil nutrients (Bakker *et al.*, 2003). The activity of grazers may also lead to a greater spatial heterogeneity of resources due to trampling or patchy removal of the herbage biomass (Bakker *et al.*, 2003) and excretion events (Betteridge *et al.*, 2010a). To date, many tools or methodologies have been developed to monitor the activities of animals (Betteridge *et al.*, 2010b; Tani *et al.*, 2013; Yoshitoshi *et al.*, 2013) and to predict their spatial distributions (Yoshitoshi *et al.*, 2015). Local plant species richness is influenced by present-day variation in grazing intensity (Klimek *et al.*, 2007) and by the historical continuity of grazing management (Johansson *et al.*, 2008).

However, assessing the diversity in plant communities from field-based data is difficult and time-consuming. Site-specific grazing management needs a high measurement density to reflect their spatial patterns within a paddock rather than an inter-paddock scale. Remote sensing is a promising tool to estimate plant productivity over large areas, and has been used to estimate grassland production. Recently, small unmanned aerial vehicles (sUAV) or drones have been introduced into agricultural researches and have become useful for monitoring plant or soil parameters on a field scale (Zhang and Kovacs, 2012). The sUAVs provide ultra-high resolution images of the plant canopy due to their low flight altitude. In contrast to satellite imagery and airborne-based remote sensing, sUAVs can be used frequently during the entire plant growing season. The main benefits are simple mission planning, instantaneous operation with low man power and imaging below the cloud cover (Floreano and Wood, 2015).

Moreover, recent advances in 3D modeling using structure-from-motion(SfM) photogrammetry have allowed researchers to utilize sUAV for initially geosciences (Smith and Vericat, 2015; Woodget *et al.*, 2015), and these methods have been expanded to various applications (Zahawi *et al.*, 2015; Cunliffe *et al.*, 2016). Although fine-grain 3D structures can be produced using sUAV-acquired image data with SfM photogrammetry (Westoby *et al.*, 2012), there have been limited applications for using this approach to characterize the biophysical structures of vegetation. Zahawi *et al.* (2015) suggested that SfM modelling of sUAV-acquired image data was not yet suitable for measuring the structure of small plants, such as grasses, due to the limitations with the accuracy of the derived canopy height models. Further refinement of the technique was needed to improve the measurement accuracy of sUAV-SfM approaches to support applications in grassland ecosystems dominated by shorter vegetation.

The aim of this review was to highlight the potential of sUAVs for monitoring spatial heterogeneity of species richness and herbage production in a grazing pasture as a case study at the Setouchi Field Science Center, Saijo Station, Graduate School of Biosphere Science, Hiroshima University, Japan (hereafter, Hiroshima University farm). The Hiroshima University farm (N34º23’, E132º43’) is located in a temperate zone with a warm, humid summer and a cool, dry winter (Lim *et al.*, 2015). The area is a boundary zone where cool-season grass or warm-season grass is grown in the recommended region. To ensure the pasture production for feeding grazing cows, the farm was using a unique strategy to combine cool-season grass (tall fescue [*Festuca arundinacea* Schreb.]) and warm-season grass (bahiagrass [*Paspalum notatum* Flűgge]) with white clover (*Trifolium repens* L.).

2. Relationship between species richness and plant productivity or nutrient status

Several studies have investigated the plant species richness–productivity relationships (Waide *et al.*, 1999; Mittelbach *et al.*, 2001; Fraser *et al.*, 2015). At Hiroshima University farm, the monthly changes in the number of species (*n*) and nutrient status data from our field survey in 2015 are shown in Fig.1, which were obtained using 0.5 m × 0.5 m quadrats on a 100 m line transect during a grazing period. Although the plant species richness (number of species) showed less or a negative relationship to herbage biomass (Kawamura *et al*. unpublished), the total digestible nutrient (TDN) concentrations tended to link the number of species.

In some cases, highly productive sites are known to be rich in resource and poor in species (Fraser *et al.*, 2015). Such high-productivity and low-diversity sites are typically highly managed via irrigation or fertilizer application and often lead to declines in the species richness relationships at high productivity. It is fact that variation in the relationship between biodiversity and ecosystem function depended on the resource availability and environmental factors (Isbell *et al.*, 2015). Particularly in grazing ecosystems, like Hiroshima University farm, grazing by large herbivores may have influenced the plant species richness–productivity. Another potential factor in Hiroshima University farm is renovation –the number of species and TDN values increased after renovation in July 13, 2015 (Fig. 1a,b).

3. Spatial distributions of herbage biomass and grazing cattle

Low-cost sUAV imagery based on an RGB consumer-level camera can compute ultra-high resolution orthoimages and surface models. There has been an increase in the use of sUAV images for estimating biophysical parameters, *e.g.*, leaf area index (LAI) (Hunt *et al.*, 2011), aboveground biomass

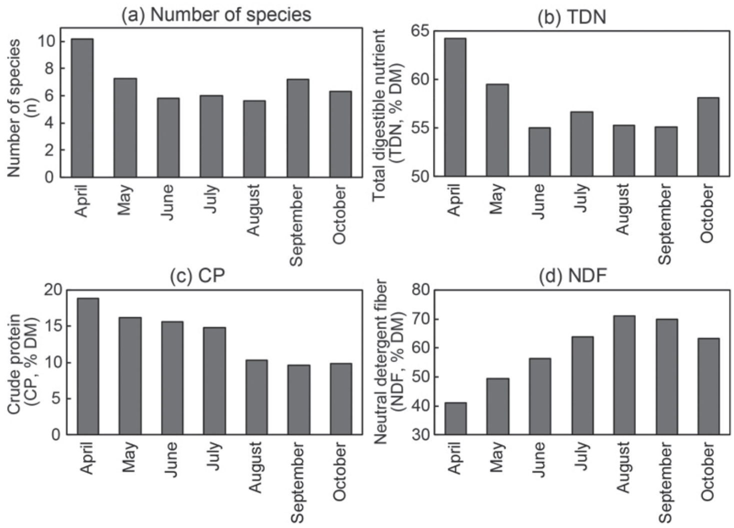

Fig. 1. Monthly changes in the number of species (*n*), total digestible nutrient (TDN), crude protein (CP) and neutral detergent fiber (NDF) concentrations of herbage from a field survey 2015 using 0.5 m × 0.5 m quadrats.

(BM) (Honkavaara *et al.*, 2013; Bendig et al., 2014) and plant height (Bendig *et al.*, 2014), as well as nitrogen (N) concentration (Lelong et al., 2008; Schirrmann *et al.*, 2016). At the Hiroshima University farm, Kawamura *et al.* (unpublished) investigated the relationship between herbage BM and the green normalized difference index (GNDVI) from sUAV images (R^2 = 0.68, $p < 0.001$) during grazing season. Using the relationship between herbage BM and the GNDVI, spatial distribution maps of BM were estimated in ultra-high spatial resolution (5 cm or more less). For example, Fig.2 shows the spatial distributions of herbage BM in prior- and post-grazing, and their differences (ΔBM) with 5 cm spatial resolution in a short-term grazing trial from 2013 (grazing period June 1–12, 2013) at Hiroshima University farm (Kawamura et al. unpublished). The spatial distributions showed that the herbage BM decreased in the southern area, while the northern areas did not change. In grazing ecosystems, large herbivores played an important role in spatial heterogeneity (Hirata *et al.*, 2011), and promoted the increase of more nutritious and palatable species. To evaluate the grazing effects, the locations of four cows fitted with GPS collars are also shown in Fig. 3. Cows were mainly grazed in the southern areas, and this approach may have also reflected the decrease in herbage BM.

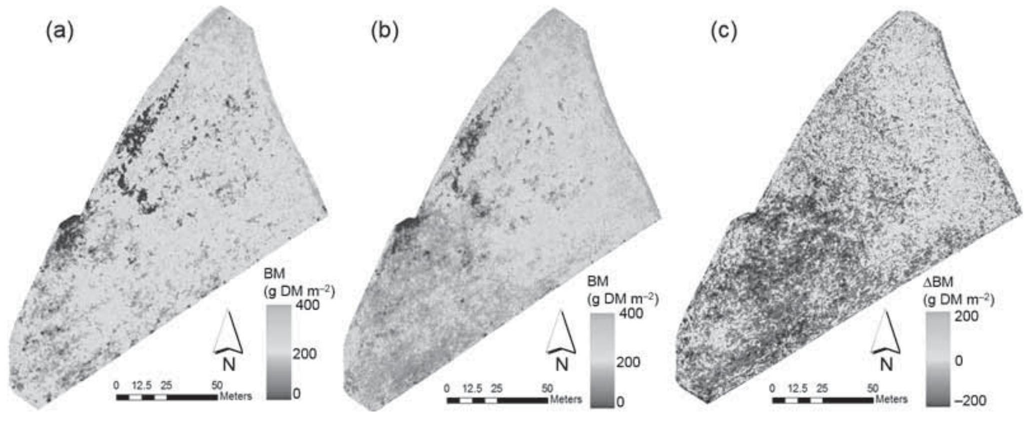

Fig. 2. Spatial distribution maps of herbage biomass (BM) in (a) prior-grazing, (b) post-grazing and (c) the difference in BM (ΔBM) between prior- and post-grazing.

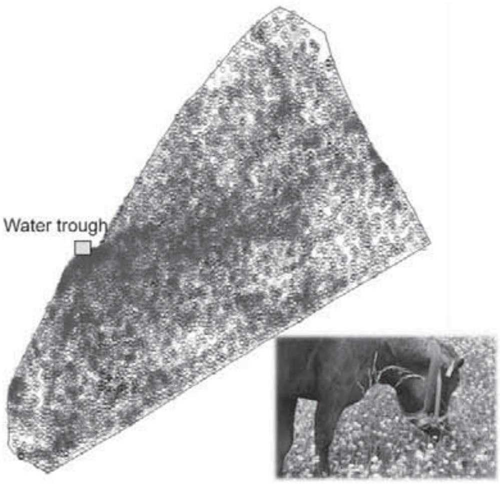

Fig. 3. Spatial distributions of the locations of four cows fitted with GPS collars.

4. Potential and limitation for an sUAV

The sUAV can be expected to be an efficient and inexpensive way to assess the productivity and the biodiversity at a paddock scale in a grazing ecosystem. This paper demonstrated (1) species richness and a productivity relationship in an artificial grassland, and (2) the potential use of an sUAV for assessing spatial distribution of herbage BM with cows' location as a case study in Hiroshima University farm.

However, similar to most technologies, an sUAV has some limitations and technical issues, including (i) payload size and weight are critical limitation factors and have trade-offs with the sensor system on an sUAV (Hunt *et al.*, 2011), (ii) stabilization may not be constant at high flight altitudes (*e.g* ., > 100 m) due to the wind being more noticeable, (iii) the battery determines the duration of the flight, and (iv) flight altitude in many countries is restricted to 120–150 m by the regulations for an sUAV (Borra-Serrano *et al.*, 2015). These limitations affect the operation planning. For example, the flight course and its altitude decide the pixel size and dimensions of the surface covered by each flight because the lower the flight altitude is, the higher the spatial resolution is but with lower surface coverage. Further research should be conducted to increase the number of potential applications for an sUAV in biodiversity and conservation studies as well as more efficient grazing management.

Acknowledgements

This study was supported by the Japan Society for the Promotion of Science (JSPS), KAKENHI (Grant numbers. 26292142 and 26850174). We are grateful to all the staff of the Setouchi Field Science Center, Saijo Station, Hiroshima University, for their assistance in the field experiments.

References

Bakker, C., JM. Blair and AK. Knapp (2003) Does resource availability, resource heterogeneity or species turnover mediate changes in plant species richness in grazed grasslands? *Oecologia* 137: 385–391.

Bendig, J., A. Bolten, S. Bennertz, J. Broscheit, S. Eichfuss and G. Bareth (2014) Estimating biomass of barley using crop surface models (CSMs) derived from UAV-based RGB imaging. *Remote Sensing*, 6: 10395–10412.

Betteridge, K., D. Costall, S. Balladur, M. Upsdell and K. Umemura (2010a) Urine distribution and grazing behaviour of female sheep and cattle grazing a steep New Zealand hill pasture.*Animal Production Science*, 50: 624–629.

Betteridge, K., C. Hoogendoorn, D. Costall, M. Carter and W. Griffiths (2010b) Sensors for detecting and logging spatial distribution of urine patches of grazing female sheep and cattle. *Computers and Electronics in Agriculture*, 73: 66–73.

Borra-Serrano, I., J. Peña, J. Torres-Sánchez, F. Mesas-Carrascosa and F. López-Granados (2015) Spatial quality evaluation of resampled unmanned aerial vehicle-imagery for weed mapping. *Sensors*, 15: 19688–19708.

Cunliffe, AM., RE. Brazier and K. Anderson (2016) Ultra-fine grain landscape-scale quantification of dryland vegetation structure with drone-acquired structure-from-motion photogrammetry. *Remote Sensing of Environment*, 183: 129–143.

Floreano, D. and RJ. Wood (2015) Science, technology and the future of small autonomous drones. *Nature*, 521: 460–466.

Fraser, LH., J. Pither, A. Jentsch, M. Sternberg, M. Zobel, D. Askarizadeh and *et al.* (2015) Worldwide evidence of a unimodal relationship between productivity and plant species richness. *Science*, 349: 302–305.

Hirata, M., M. Higashiyama and N. Hasegawa (2011) Diurnal pattern of excretion in grazing cattle. *Livestock Science*, 142: 23–32.

Honkavaara, E., H. Saari, J. Kaivosoja, I. Pölönen,

T. Hakala, P. Litkey, J. Mäkynen and L. Pesonen (2013) Processing and assessment of spectrometric, stereoscopic imagery collected using a lightweight UAV spectral camera for precision agriculture. *Remote Sensing*, 5: 5006–5039.

Hunt, E., W. Hively, G. McCarty, C. Daughtry, P. Forrestal, R. Kratochvil, J. Carr, N. Allen, J. Fox-Rabinovitz and C. Miller (2011) NIR-green-blue high-resolution digital images for assessment of winter cover crop biomass. *Giscience & Remote Sensing*, 48: 86–98.

Isbell, F., D. Craven, J. Connolly, M. Loreau, B. Schmid, C. Beierkuhnlein and et al. (2015) Biodiversity increases the resistance of ecosystem productivity to climate extremes. *Nature*, 526: 574–577.

Johansson, LJ., K. Hall, HC. Prentice, M. Ihse, T. Reitalu, MT. Sykes and M. Kindström (2008) Semi-natural grassland continuity, long-term land-use change and plant species richness in an agricultural landscape on Öland, Sweden. *Landscape and Urban Planning*, 84: 200–211.

Klimek, S., AR. Kemmermann, M. Hofmann and J. Isselstein (2007) Plant species richness and composition in managed grasslands: The relative importance of field management and environmental factors.*Biological Conservation*, 134: 559–570

Lelong, C., P. Burger, G. Jubelin, B. Roux, S. Labbé and F. Baret (2008) Assessment of unmanned aerial vehicles imagery for quantitative monitoring of wheat crop in small plots. *Sensors*, 8: 3557–3585.

Lim, J., K. Kawamura, HJ. Lee, R. Yoshitoshi, Y. Kurokawa, Y. Tsumiyama and N. Watanabe (2015) Evaluating a hand-held crop-measuring device for estimating the herbage biomass, leaf area index and crude protein content in an Italian ryegrass field. *Grassland Science*, 61: 101–108.

Mittelbach, GG., CF. Steiner, SM. Scheiner, KL. Gross, HL. Reynolds, RB. Waide, MR. Willig, SI. Dodson and L. Gough (2001) What is the observed relationship between species richness and productivity? Ecology, 82: 2381–2396.

Mizuno, H., Y. Yoshihara, T. Inoue, K. Kimura, S. Tanaka, S. Sato and S. Ogura (2012) The effect of species richness of vegetation on mineral condition of grazing cattle in a Japanese alpine pasture. *Proceedings of the 4th Japan-China-Korea Grassland Congress*, pp.242–243.

Ogura, S. (2011) Diet selection and foraging behavior of cattle in species-rich vegetation of native grasslands, Japan. *Journal of Integrated Field Science,* 8: 25–33.

Schirrmann, M., A. Giebel, F. Gleiniger, M. Pflanz, J. Lentschke and KH. Dammer (2016) Monitoring Agronomic Parameters of Winter Wheat Crops with Low-Cost UAV Imagery. *Remote Sensing* 8, 706. DOI: 10.3390/rs8090706

Smith, MW. and D. Vericat (2015) From experimental plots to experimental landscapes: topography, erosion and deposition in sub-humid badlands from Structure-from-Motion photogrammetry. *Earth Surface Processes and Landforms*, 40: 1656–1671.

Takamizawa, S., T. Shishido and S. Ogura (2016b) Effect of native grasses, forbs and trees on nutrient uptake of grazing cattle in a temperate region of Japan. *Proceedings of 10th International Rangeland Congress*, pp.1174–1176.

Tani, Y., Y. Yokota, M. Yayota and S. Ohtani (2013) Automatic recognition and classification of cattle chewing activity by an acoustic monitoring method with a single-axis acceleration sensor. *Computers and Electronics in Agriculture*, 92: 54–65.

Waide, RB., MR. Willig, CF. Steiner, G. Mittelbach, L. Gough, SL. Dodson, GP. Juday and R. Parmenter (1999) The relationship between productivity and species richness. *Annual Review of Ecology and Systematics*, 30: 257–300.

Wang, R., J. Gamon, R. Montgomery, P. Townsend, A. Zygielbaum, K. Bitan, D. Tilman and J. Cavender-Bares (2016) Seasonal variation in the NDVI–species richness relationship in a Prairie grassland experiment (Cedar Creek). *Remote Sensing* 8, 128. DOI:10.3390/rs8020128

Westoby, MJ., J. Brasington, NF. Glasser, MJ. Hambrey and JM. Reynolds (2012) ‘Structure-from-Motion’ photogrammetry: A low-cost, effective tool for geoscience applications. *Geomorphology,* 179: 300–314.

Woodget, AS., PE. Carbonneau, F. Visser and IP. Maddock (2015) Quantifying submerged fluvial topography using hyperspatial resolution UAS imagery and structure from motion photogrammetry. *Earth Surface Processes and Landforms*, 40: 47–64.

Yoshitoshi, R., N. Watanabe, K. Kawamura, S. Sakanoue, R. Mizoguchi, HJ. Lee and Y. Kurokawa (2013) Distinguishing cattle foraging activities using an accelerometry-based activity monitor. *Rangeland Ecology & Management*, 66: 382–386.

Yoshitoshi, R., N. Watanabe, T. Yasuda, K. Kawamura, S. Sakanoue, J. Lim and HJ. Lee (2015) Preliminary study to predict the spatial distribution of dung from beef cattle in a slope-grazed pasture. *Grassland Science*, 61: 50–55.

Zahawi, RA., JP. Dandois, KD. Holl, D. Nadwodny, JL. Reid and EC. Ellis (2015) Using lightweight unmanned aerial vehicles to monitor tropical forest recovery. *Biological Conservation*, 186: 287–295.

Zhang, C. and JM. Kovacs (2012) The application of small unmanned aerial systems for precision agriculture: a review. *Precision Agriculture*, 13: 693–712.

JIFS, 14 : 67 − 75 (2017)

Hyperspectral Assessment for Legume Content and Forage Nutrient Status in Pastures

Jihyun LIM[1,*], Kensuke KAWAMURA[2], Rena YOSHITOSHI1, Masato YAYOTA[3] and Shin-ichiro OGURA[4]

[1]Global Career Design Center, Hiroshima University, Japan
[2]International Research Center for Agricultural Sciences (JIRCAS), Japan
[3]Faculty of Applied Biological Science, Gifu University, Japan
[4]Graduate School of Agricultural Science, Tohoku University, Japan

Correspondence: Jihyun LIM
Tel: +81-82-424-4588
e-mail: limjihyun7@gmail.com

Keywords: grazing management, hyperspectral remote sensing, legume content, partial least squares (PLS) regression, vegetation indices (VIs)

Abstract

The spectral assessment of pasture biomass and nutrient status is influenced by floristic composition. The accurate estimation of the nutrient status in a pasture throughout the growing season is challenging and a critical step to establish a site-specific management strategy for the improvement of productivity and profitability as well as the mitigation of the environmental impact. Remote sensing technologies have been widely applied to vegetation surveys because they can quickly retrieve the *in situ* biophysical and biochemical information of a field. Recent advances in sensing technologies, especially in a hyperspectral sensor system that has a higher spectral resolution of less than 10-nm bandwidth, have significantly improved predictive ability for the estimated biomass quantification in comparison with the conventional broad-band sensor system. Not only the biomass quantification but also other information about the pasture, such as forage nutrient content and the floristic composition, can also be estimated using its abundant spectral information, which is difficult to achieve on a broad-band sensor system. In this mini-review, we discuss the use of hyperspectral assessment to estimate the forage parameters of a pasture. Recent improvements in the analysis methodology of hyperspectral data have been reviewed and include (i) a univariate statistical approach based on narrow-band vegetation indices, (ii) multivariate statistical approaches, especially using partial least squares (PLS) regression, (iii) waveband selection to enhance the predictive performance of PLS regression, and (iv) the spatial interpolation of predicted values from ground-based hyperspectral measurements.

1. Introduction

Grassland ecosystems have spatial and temporal dynamics in the biotic factors such as floristic composition, forage nutrients (*e.g.*, protein, minerals and energy) and plant productivity (Bailey *et al.*, 1996; Vallentine, 2000), and there are interactions between the dynamics and the grazing distribution of animals during the growing seasons with abiotic factors such as the slope, elevation, aspect (Govender *et al.*, 2007) and distance from water sources (Yoshitoshi *et al.*, 2016). The timely and accurate quantification of the biotic factors in a grazed paddock, particularly the biophysical and biochemical characteristics of the pasture and its spatial distribution are essential to facilitate the decision-making process to enable adequate agronomic operation, such as controlling the grazing intensity, adjusting the fertilizer level and determining the best time for mowing, throughout the growing season.

Remote sensing techniques provide such information on vegetation in rangeland and agricultural crop fields in a non-destructive, quick, and inexpensive manner compared to the conventional destructive method such as wet chemistry; they also require less labor and have reduced environmental impacts.

Remote sensing techniques using the optical region that includes the visible and near-infrared (NIR) portions of the electromagnetic spectrum have been successfully used to quantify the biophysical characteristics of vegetation based on their optical properties in past decades (Gates et al., 1965; Allen et al., 1969; Tucker, 1980; Gamon et al., 1995; Cohen et al., 2003). However, these conventional broadband remote sensing approaches for estimating the vegetation biochemical characteristics within the optical region have a limitation due to their lower spectral resolution, which leads to the loss of critical vegetation information associated with the absorption features located in specific narrow bands (Curran, 1989; Blackburn, 1998; Thenkabail et al., 2000). Recent advances in sensing technologies in the spectral resolution, from multispectral sensors to hyperspectral sensors with less than 10-nm spectral resolution (Asner, 1998), enable the detection of narrow absorption features (Fig. 1), and allow more accurate quantification of not only the biophysical characteristics of the vegetation in the pasture but also other information. Recently, it has been demonstrated that ground-based hyperspectral measurement can estimate the floristic composition such as legume content (Biewer et al., 2009; Sanches, 2009; Kawamura et al., 2013) and forage nutrients of a pasture (Mutanga and Kumar, 2007; Zhao et al., 2007; Kawamura et al., 2009; Pullanagari et al., 2012,) with improved temporal frequency and lower cost than spaceborne or airborne instruments, and such enhanced performance highlights the spatial variability of the pasture characteristics within a field as well as differences between fields (Kawamura et al., 2008a, Lim et al., 2015b).

This review describes the necessity and potential of field hyperspectral assessment for the estimation of forage nutrient status and legume content in the pasture. Recent improvements in the analytical methodology of hyperspectral data are described based on statistical approaches, and widely applied methods are described. For this aim, (i) a univariate statistical approach using narrow band vegetation indices, (ii) multivariate statistical approaches, especially using partial least squares (PLS) regression, (iii) waveband selection to enhance the predictive performance of PLS regression and (iv) the spatial interpolation of predicted values from field hyperspectral measurements were described.

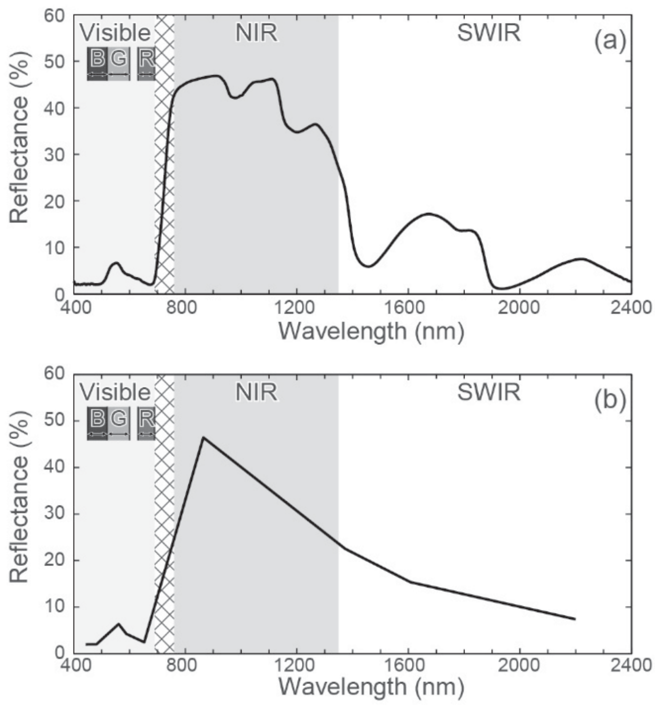

Fig.1. (a) Hyperspectral reflectance of healthy mixed-sown pasture (average canopy reflectance of grazing pasture measured by an ASD FieldSpec Pro radiometer) in the optical region ranging from visible (blue (B), green (G) and red (R)) and near-infrared (NIR) to short-wave infrared (SWIR) and the spectral response with the Landsat 8 band-setting as an example of broad-band remote sensing.

2. Narrow vegetation indices

One of the most common approaches to estimate the vegetation properties from remotely sensed data is making an empirical regression model. Such a statistical approach involves univariate or multivariate regression analysis. In conventional broadband remote sensing, vegetation indices (VIs), which are computations of the spectral response in two or more bands such as simple ratios (SR) and the normalized difference vegetation index (NDVI) (Rouse *et al.*, 1974), have been widely applied to find relations between the characteristics of vegetation via univariate regression analysis. To date, various VIs have been developed and successfully used to quantify plant biophysical properties (Rouse *et al.*, 1974; Huete, 1988; Roujean and Breon, 1995; Chen, 1996; Gitelson *et al.*, 1996). Most of these conventional VIs are based on the contrast between two or more spectral bands. For example, NDVI, one of the most widely used VIs, defined as (R_{NIR}-R_{red}) / (R_{NIR}+R_{red}), uses low reflectance in the red band related to chlorophyll absorption and high reflectance in the NIR due to multiple scattering effects to predict the greenness of vegetation (Rouse *et al.*, 1974). However, NDVI approaches generally saturate asymptotically under conditions of moderate to high biomass (Gitelson, 2004, Lim *et al.*, 2015a) due to decreasing sensitivity at the NIR band, which faces difficulty related to the distinguishability of the temporal and spatial variabilities of the pasture characteristics in the different growing stages.

Considerable efforts have been expanded to find new combinations of narrow bands of NDVI (Mutanga and Skidmore, 2004b; Cho *et al.*, 2007; Kawamura *et al.*, 2011) and SR (Fava *et al.*, 2009) derived data from hyperspectral measurements. These new combinations of VIs have exhibited improved predictive ability for quantifying biophysical and biochemical variables than the conventional red-NIR band combination in the grassland environment. It has been reported that the critical waveband combinations vary with the parameters (Mutanga and Skidmore, 2004b; Cho *et al.*, 2007; Kawamura *et al.*, 2011; Lim *et al.*, 2012) and growth stage (Fava *et al.*, 2009) ,which suggests the potentials to develop parameter specific indices (Gamon *et al.*, 1992). For instance, an experiment conducted on a sheep-grazed pasture exhibiting various levels of fertility (Betteridge *et al.*, 2010) (n = 25) using the narrow-band normalized difference spectral index (NDSI), based on the traditional equation of NDVI defined as (R_{Band1}–R_{Band2}) / (R_{Band1}+R_{Band2}), demonstrated much better performance in estimating the herbage biomass (R^2 = 0.42 to 0.83) and the concentrations (% of dry matter [DM]) of nitrogen (N) (R^2 = 0.46 to 0.73) and phosphorus (P) (R^2 = 0.11 to 0.86) than the standard NDVI as measured by the coefficient of determination (R^2) values using hyperspectral measurements ranging from 400 to 2500 nm. The wavelength regions that were regarded as critical, showing higher R^2 values, for estimating the pasture properties were different in each parameter (Fig. 2), and this showed better predictive accuracy than the red-NIR combination which is generally employed in broad-band VIs.

3. Multivariate statistical approach - partial least squares (PLS) analysis

Multivariate statistical approaches have been proposed to utilize high spectral dimensionality such as multiple linear regression (MLR) (Curran, 1989; Kokaly and Clark, 1999; Zhao *et al.*, 2007), principal component regression (PCR) (Peñuelas *et al.*,

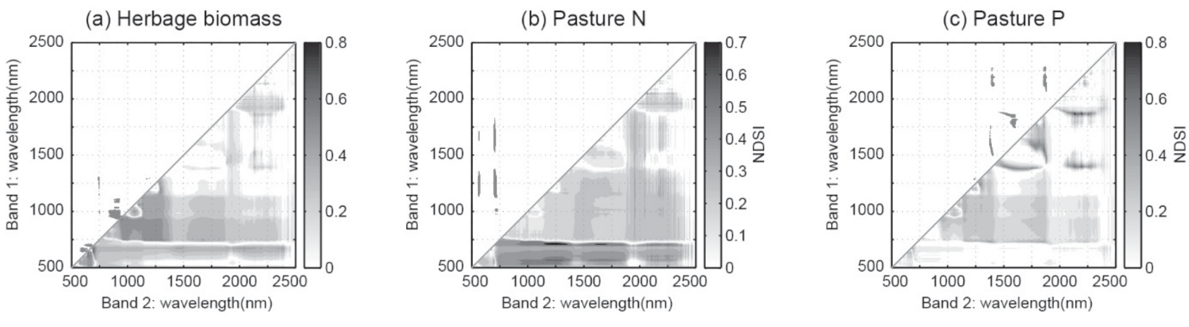

Fig.2. Narrow band normalized-difference spectral index (NDSI) and critical wavelength region to estimate (a) herbage biomass and concentrations of (b) nitrogen and (c) phosphorous of a sheep-grazed paddock (n = 25).

1993),partial least squares (PLS) regression (Geladi and Kowalski, 1986; Hansen and Schjoerring, 2003), support vector machines (SVMs) (Vapnik, 2013), and neural networks (Mutanga and Skidmore, 2004a) using the original hyperspectral response or transformations of the spectra such as first derivative reflectance (FDR) (Dawson and Curran, 1998, Kawamura *et al.*, 2010) and continuum removed absorption features (CRDR) (Mutanga *et al.*, 2004). These approaches have demonstrated their potential for vegetation parameter estimation. PLS regression is the most widely used bi-linear method and is able to include all available waveband information in the model (Wold *et al.*, 2001) due to its superiority for multi-collinear data processing. PLS regression is a fundamental method that has long been used in laboratory near-infrared spectroscopy (NIRS) calibration and has been increasingly used in - field assessment of forage quantity and quality parameters such as crude protein (CP = N×6.24), metabolic energy, acid detergent fiber (ADF) and reported good performance (Schut et al., 2005; Schut et al., 2006; Zhao et al., 2007; Biewer et al., 2009b; Pullanagari et al., 2012).

3.1 Wavebands selection to improve the predictive ability of the PLS model

The complete canopy spectra of the in situ vegetation contains redundant information such as the mechanical noise, soil background effects and water absorption in the atmosphere (Gates *et al.*, 1965; Woolley, 1971; Vanderbilt *et al.*, 1985). Moreover, some researchers have reported that no significant improvement in the vegetation parameter estimations was discovered in a comparative study with PLS using the full spectrum (FS-PLS) between the optimized narrow-band VIs for rice (Nguyen and Lee, 2006; Inoue *et al.*, 2008) and wheat (Hansen and Schjoerring, 2003). Recently, developing a PLS model with wavelength region selection has been regarded as a promising way to improve the prediction power of the model (Darvishzadeha *et al.*, 2008). To date, various approaches have been developed to eliminate useless wavebands or to select informative wavebands, such as moving-window PLS (MW-PLS) (Jiang *et al.*, 2002), iterative stepwise elimination PLS (ISE-PLS), uninformative variable elimination PLS (UVE-PLS) (Centner *et al.*, 1996), and genetic algorithm PLS (GA-PLS) (Leardi *et al.*, 1992).

There was significant improvement of the PLS model with spectral subset selection to estimate the pasture nutritional quality as well as biomass (*e.g.*, Fig. 3). The concentrations of the macronutrients (*e.g.*, phosphorus, potassium, calcium and magnesium) which are mainly responsible for the plant development and determine the forage nutritional quality (Schmidtlein and Sassin, 2004; Darvishzadeha *et al.*, 2008; Kawamura *et al.*, 2008b; Kawamura *et al.*, 2010), fiber (Kawamura *et al.*, 2010) and legume content (Kawamura *et al.*, 2013) can be estimated using the *in situ* hyperspectral spectra of the pasture. Kawamura et al. (2013) reported that only less than 10% of the wavebands remain from the complete canopy reflectance data (400–2350 nm) to discriminate legumes, and less than 20% of wavebands are used for the determining the forage nutritional concentration in the experiment conducted on cattle-grazed pasture of different growth stages. This suggests that the complete spectral information with high dimensionality also contains redundant information that can be a disturbance or otherwise not contribute to the estimation of the pasture characteristics.

In spite of the agreement and demands to the developing the integrated calibration model of field hyperspectral measurement for the assessment of forage quality and floristic composition of pasture, further investigation to clarify the critical wavelength region for each forage parameter and application to multivariate statistical approaches such as PLS is still required. Past studies have been devoted to clarify the wavelength regions which are attributed from photosynthetic pigment absorption in visible region to red-edge such as chlorophyll centered on 430, 460, 520, 550, 640, 680 and 690 nm (Curran, 1989; Chappelle et al., 1992; Thenkabail et al., 2004; Chan and Paelinckx, 2008). The red-edge region (Horler et al., 1983; Peñuelas et al., 1993; Thenkabail et al., 2000; le Maire et al., 2008; Chan and Paelinckx, 2008) which is strongly associated with protein (N) and carotenoids (Car) bands is related to physiological status centered on 470 nm (Blackburn, 1998) and 510 nm (Gitelson *et al.*, 1996). More consideration should be demonstrated to determine the wavelength region for macronutrients (e.g., phosphorus, potassium, calcium and magnesium) known as potentially located in short-wave infrared region (Mutanga and Kumar, 2007; Pimstein *et al.*, 2011; Ramoelo *et al.*, 2011).

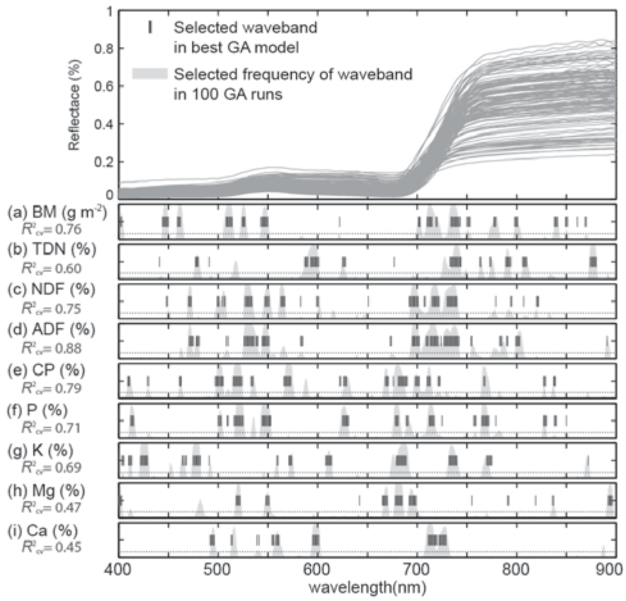

Fig. 3. Selected wavebands by the best GA-PLS model in 100 runs (black line) and the selection frequency of each wavelength by 100 runs of GA-PLS (grey solid) to estimate the herbage biomass (BM) and concentration of total digestible nutrients (TDN), neutral detergent fiber (NDF), acid detergent fiber (ADF), crude protein (CP), phosphorus (P), potassium (K), magnesium (Mg) and calcium (Ca) using the genetic algorithm partial least squares (GA-PLS) and cross-validated R^2 in a cool-season Italian ryegrass meadow field. (Edited from Lim et al)

3.2 Application: Spatial interpolation of field hyperspectral assessment

Lim *et al*. (2015b) reported that the improved prediction power of the GA-PLS model (Fig. 3) resulted in the efficient elimination of redundant spectral information. Using this GA-PLS model, forage parameters (*e.g.*, total digestible nutrients (TDN)) were estimated non-destructively based on the in-field canopy hyperspectral measurement data collected with 10m interval (Lim *et al.*, 2015a) and the map generated by the spatial interpolation of the estimated parameters. From the map, the growth development and nutritive status of the grass can be monitored throughout the two consecutive growing seasons. Especially nutritive changes of pasture such as the timing to leach the peak of nutritive values and its spatial and temporal distributions in the Italian ryegrass field have been distinguished within filed inherently as well as between fields and seasons (Fig. 4) under different fertilization controls (Lim *et al.*, 2015a). The results suggest the possibility of 'real-time' monitoring of the pasture, especially forage nutritive values which is invisible, troughout a growing season without destructive manners. Such information may contribute to determining the timing for maintaining the fertility level or cutting with fine-scale to produce high-quality forage during the growing season in real time.

4. Potential of Hyperspectral Assessment for Legume Content and Forage Nutrient Status in Pasture

Field hyperspectral assessment has enabled significant advances for determining the forage nutrient concentration and discriminating the floristic composition (*e.g.*, legume) of a pasture as well as biomass refining informative wavelength region approaches with narrow-band hyperspectral VIs and multivariate statistical approaches (*e.g.*, PLS) quickly and non-destructively. However, limited studies have been performed to determine the critical wavelength region to estimate the pasture characteristics, especially the concentrations of forage nutrients (*e.g.*, P, K, calcium and energy), and legumes and the results are time- and site-specific, except for biomass and nitrogen which are known to be strongly associated with chlorophyll content. More efforts should be made to define the waveband and its integral application on various grassland environments.

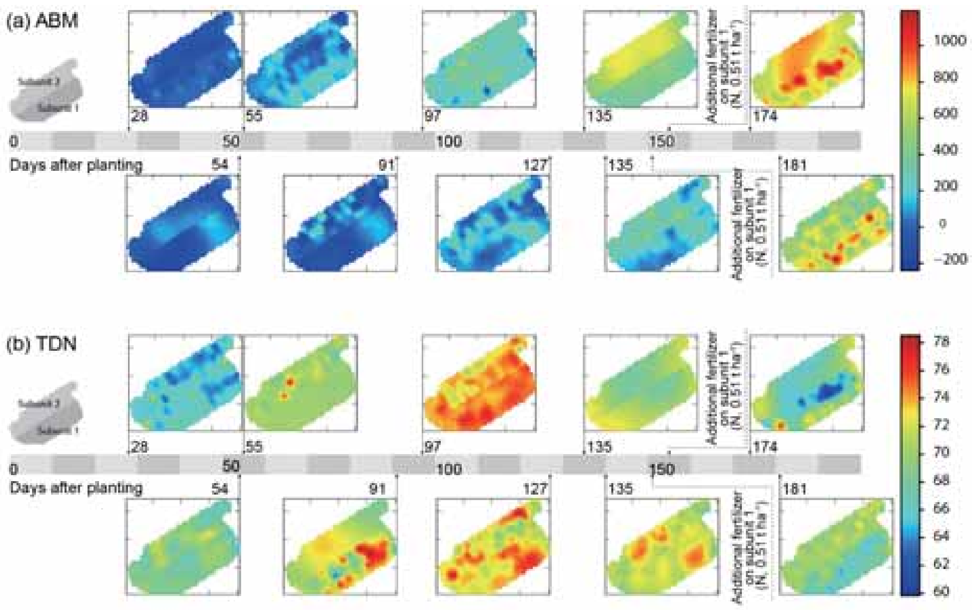

Fig. 4. Spatial distribution maps (5 m grid) of an Italian ryegrass meadow field obtained by ordinary kriging for (a) ABM, (b) NDF, (c) TDN and (d) CP by days after planting (DAF) (Lim *et al.*, 2015b).

Acknowledgements

This study was partially supported by the Japan Society for the Promotion of Science (JSPS), KAKENHI (Grant number. 26850174). We are grateful to all the staff of the Setouchi Field Science Center, Saijo Station, Hiroshima University, and AgResearch, New Zealand for their assistance in the field experiments.

References

Allen, W.A., H.W. Gausman, A.J. Richardson and J.R. Thomas (1969) Interaction of isotropic light with a compact plant leaf. JOSA, 59: 1376–1379.

Asner, G.P. (1998) Biophysical and biochemical sources of variability in canopy reflectance. Remote Sensing of Environment, 64: 234–253.

Bailey, D.W., J.E. Gross, E.A. Laca and et al. (1996) Mechanisms that result in large herbivore grazing distribution patterns. Journal of Range Management, 49: 386–400.

Betteridge, K., D. Costall, S. Balladur, M. Upsdell and K. Umemura (2010) Urine distribution and grazing behaviour of female sheep and cattle grazing a steep New Zealand hill pasture. Animal Production Science, 50: 624–629.

Biewer, S., S. Erasmi, T. Fricke and M. Wachendorf (2009a) Prediction of yield and the contribution of legumes in legume-grass mixtures using field spectrometry. Precision Agriculture, 10: 128–144.

Biewer, S., T. Fricke and M. Wachendorf (2009b) Development of canopy reflectance models to predict forage quality of legume-grass mixtures. Crop Science, 49: 1917–1926.

Blackburn, G.A. (1998) Quantifying chlorophylls and caroteniods at leaf and canopy scales: An evaluation of some hyperspectral approaches. Remote Sensing of Environment, 66: 273–285.

Centner, V., D.L. Massart, O.E. De Noord, S. De Jong, B.M. Vandeginste and C. Sterna (1996) Elimination of uninformative variables for multivariate calibration. Analytical Chemistry, 68: 3851–3858.

Chan, J.C.W. and D. Paelinckx (2008) Evaluation of Random Forest and Adaboost tree-based ensemble classification and spectral band selection for ecotope mapping using airborne hyperspectral imagery. Remote Sensing of Environment, 112: 2999–3011.

Chappelle, E.W., M.S. Kim and J.E. Mcmurtrey (1992) Ratio analysis of reflectance spectra (RARS): an algorithm for the remote estimation of the concentrations of chlorophyll a, chlorophyll b, and carotenoids in soybean leaves. Remote Sensing of Environment, 39: 239–247.

Chen, J.M. (1996) Evaluation of vegetation indices and a modified simple ratio for boreal applications. Canadian Journal of Remote Sensing, 22: 229–242.

Cho, M.A., A.K. Skidmore, F. Corsi, S.E. Van Wieren and I. Sobhan (2007) Estimation of green grass/herb biomass from airborne hyperspectral imagery using spectral indices and partial least squares regression. International Journal of Applied Earth Observation and Geoinformation, 9: 414–424.

Cohen, W.B., T.K. Maiersperger, S.T. Gower and D.P. Turner (2003) An improved strategy for regression of biophysical variables and Landsat ETM+ data. Remote Sensing of Environment, 84: 561–571.

Curran, P.J. (1989) Remote sensing of foliar chemistry. Remote Sensing of Environment, 30: 271–278.

Darvishzadeha, R., A.K. Skidmore, M. Schlerf, C. Atzberger, F. Corsi and M. Cho (2008) LAI and chlorophyll estimation for a heterogeneous grassland using hyperspectral measurements. ISPRS Journal of Photogrammetry and Remote Sensing, 63: 409–426.

Dawson, T. and P. Curran (1998) Technical note. A new technique for interpolating the reflectance red edge position.

Fava, F., R. Colombo, S. Bocchi and et al. (2009) Identification of hyperspectral vegetation indices for Mediterranean pasture characterization. International journal of applied earth observation and geoinformation, 11: 233–243.

Gamon, J., J. Penuelas and C. Field (1992) A narrow-waveband spectral index that tracks diurnal changes in photosynthetic efficiency. Remote Sensing of Environment, 41: 35–44.

Gamon, J., C.B. Field, M.L. Goulden and et al. (1995) Relationships between NDVI, canopy structure, and photosynthesis in three californian vegetation types. Ecological Applications, 5: 28–41.

Gates, D.M., H.J. Keegan, J.C. Schleter and V.R. Weidner (1965) Spectral properties of plants. Applied Optics, 4: 11–20.

Geladi, P. and B.R. Kowalski (1986) Partial least-squares regression: a tutorial. Analytica Chimica Acta, 185: 1–17.

Gitelson, A.A. (2004) Wide dynamic range vegetation index for remote quantification of biophysical characteristics of vegetation. Journal of Plant Physiology, 161: 165–173.

Gitelson, A.A., Y.J. Kaufman and M.N. Merzlyak (1996) Use of a green channel in remote sensing of global vegetation from EOS-MODIS. Remote Sensing of Environment, 58: 289–298.

Govender, M., K. Chetty and H. Bulcock (2007) A review of hyperspectral remote sensing and its application in vegetation and water resource studies. Water Sa, 33.

Hansen, P.M. and J.K. Schjoerring (2003) Reflectance measurement of canopy biomass and nitrogen status in wheat crops using normalized difference vegetation indices and partial least squares regression. Remote Sensing of Environment, 86: 542–553.

Horler, D.N.H., M. Dockray and J. Barber (1983) The red edge of plant leaf reflectance. International Journal of Remote Sensing, 4: 273–288.

Huete, A.R. (1988) A soil-adjusted vegetation index (SAVI). Remote Sensing of Environment, 25: 295–309.

Inoue, Y., G. Miah, E. Sakaiya, K. Kaneko and K. Kawamura (2008) NDSI map and IPLS using hyperspectral data for assessment of plant and ecosystem variable : With a case study on remote sensing of grain protein content, chloropyll content and biomass in rice. Journal of the Remote Sensing Society of Japan, 28: 317–330.

Jiang, J.H., R.J. Berry, H.W. Siesler and Y. Ozaki (2002) Wavelength interval selection in multicomponent spectral analysis by moving window partial least-squares regression with applications to mid-infrared and near-infrared spectroscopic data. Analytical Chemistry, 74: 3555–3565.

Kawamura, K., K. Betteridge, D. Costall, I.D. Sanches, M. Tuohy and Y. Inoue (2008a) Hyperspectral canopy reflectance as a potential tool to estimate and map pasture mass and quality.

Kawamura, K., K. Betteridge, I.D. Sanches, M.P. Tuohy, D. Costall and Y. Inoue (2009) Field radiometer with canopy pasture probe as a potential tool to estimate and map pasture biomass and mineral components: a case study in the Lake Taupo catchment, New Zealand. New Zealand Journal of Agricultural Research, 52: 417–434.

Kawamura, K., A.D. Mackay, M.P. Tuohy, K. Betteridge, I.D. Sanches and Y. Inoue (2011) Potential for spectral indices to remotely sense phosphorus and potassium content of legume-based pasture as a means of assessing soil phosphorus and potassium fertility status. International Journal of Remote Sensing, 32: 103–124.

Kawamura, K., N. Watanabe, S. Sakanoue and Y. Inoue (2008b) Estimating forage biomass and quality in a mixed sown pasture based on PLS regression with waveband selection. Grassland Science, 54: 131–146.

Kawamura, K., N. Watanabe, S. Sakanoue, H.J. Lee, Y. Inoue and S. Odagawa (2010) Testing genetic algorithm as a tool to select relevant wavebands from field hyperspectral data for estimating pasture mass and quality in a mixed sown pasture using partial least squares regression. Grassland Science, 56: 205–216.

Kawamura, K., N. Watanabe, S. Sakanoue, H.J. Lee, J. Lim and R. Yoshitoshi (2013) Genetic algorithm-based partial least squares regression for estimating legume content in a grass-legume mixture using field hyperspectral measurements. Grassland Science, 59: 166–172.

Kokaly, R.F. and R.N. Clark (1999) Spectroscopic determination of leaf biochemistry using band-depth analysis of absorption features and stepwise multiple linear regression. Remote Sensing of Environment, 67: 267–287.

Leardi, R., R. Boggia and M. Terrile (1992) Genetic algorithms as a strategy for feature selection. Journal of Chemometrics, 6: 267–281.

Le Maire, G., C. François, K. Soudani and et al. (2008) Calibration and validation of hyperspectral indices for the estimation of broadleaved forest leaf chlorophyll content, leaf mass per area, leaf area index and leaf canopy biomass. Remote Sensing of Environment, 112: 3846–3864.

Lim, J., K. Kawamura, H.J. Lee and et al. (2015a) Evaluating a hand-held crop-measuring device for estimating the herbage biomass, leaf area index and crude protein content in an Italian ryegrass field. Grassland Science, 61: 101–108.

Lim, J., K. Kawamura, R. Yoshitoshi, K. Betteridge and D. Costall (2012) "Hyperspectral proximal sensing as a tool for estimating herbage biomass and forage nutrient status in grassland, New Zealand". Proceeding of The 3rd Australian and New Zealand Spatially Enabled Livestock Management Symposium: 13–14.

Lim, J., K. Kawamura, R. Yoshitoshi, Y. Kurokawa and N. Watanabe (2015b) Mapping forage quantity and quality in Italian ryegrass field during growing season using proximal sensing. Grassland Sicences, 61(ext.): 2.

Mutanga, O. and L. Kumar (2007) Estimating and mapping grass phosphorus concentration in an African savanna using hyperspectral image data. International Journal of Remote Sensing, 28: 4897–4911.

Mutanga, O. and A.K. Skidmore (2004a) Integrating imaging spectroscopy and neural networks to map grass quality in the Kruger National Park, South Africa. Remote Sensing of Environment, 90: 104–115.

Mutanga, O. and A.K. Skidmore (2004b) Narrow band vegetation indices overcome the saturation problem in biomass estimation. International Journal of Remote Sensing, 25: 3999–4014.

Mutanga, O., A.K. Skidmore and H. Prins (2004) Predicting in situ pasture quality in the Kruger National Park, South Africa, using continuum-removed absorption features. Remote Sensing of Environment, 89: 393–408.

Mutanga, O. and L. Kumar (2007) Estimating and mapping grass phosphorus concentration in an African savanna using hyperspectral image data. International Journal of Remote Sensing, 28: 4897–4911.

Nguyen, H.T. and B.W. Lee (2006) Assessment of rice leaf growth and nitrogen status by hyperspectral canopy reflectance and partial least square regression. European Journal of Agronomy, 24: 349–356.

Peñuelas, J., J.A. Gamon, K.L. Griffin and C.B. Field (1993) Assessing community type, plant biomass, pigment composition, and photosynthetic efficiency of aquatic vegetation from spectral reflectance. Remote Sensing of Environment, 46: 110–118.

Pimstein, A., A. Karnieli, S.K. Bansal and D.J. Bonfil (2011) Exploring remotely sensed technologies for monitoring wheat potassium and phosphorus using field spectroscopy. Field Crops Research, 121: 125–135.

Pullanagari, R.R., I.J. Yule, M.P. Tuohy, M. Hedley, R.A. Dynes and W.M. King (2012) In-field hyperspectral proximal sensing for estimating quality parameters of mixed pasture. Precision Agriculture, 13: 351–369.

Ramoelo, A., A.K. Skidmore, M. Schlerf, R. Mathieu and I.M.A. Heitkönig (2011) Water-removed spectra increase the retrieval accuracy when estimating savanna grass nitrogen and phosphorus concentrations. ISPRS Journal of Photogrammetry and Remote Sensing, 66: 408–417.

Roujean, J.L. and F.M. Breon (1995) Estimating PAR absorbed by vegetation from bidirectional reflectance measurements. Remote Sensing of Environment, 51: 375–384.

Rouse, J.W., R.H. Haas, J.A. Schell and D.W. Deering (1974) Monitoring vegetation systems in the Great Plains with ERTS. In S. C. Freden, E. P. Mercanti, & M. Becker (Eds.), Third Earth Resources Technology Satellite–1 Symposium. NASA SP-353, 1: 309–317.

Sanches, I.D.A. (2009) Hyperspectral proximal sensing of the botanical composition and nutrient content of New Zealand pastures: a thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Earth Science. In: Hyperspectral proximal sensing of the botanical composition and nutrient content of New Zealand pastures: a thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Earth Science. Massey University, Palmerston North, New Zealand, 219

Schmidtlein, S. and J. Sassin (2004) Mapping of continuous floristic gradients in grasslands using hyperspectral imagery. Remote Sensing of Environment, 92: 126–138.

Schut, A.G.T., C. Lokhorst, M. Hendriks, J. Kornet and G. Kasper (2005) Potential of imaging spectroscopy as tool for pasture management. Grass and Forage Science, 60: 34–45.

Schut, A.G.T., G.W.a.M. Van Der Heijden, I. Hoving, M.W.J. Stienezen, F.K. Van Evert and J. Meuleman (2006) Imaging spectroscopy for on-farm measurement of grassland yield and quality. Agronomy Journal, 98: 1318–1325.

Thenkabail, P.S., R.B. Smith and E. De Pauw (2000) Hyperspectral vegetation indices and their relationships with agricultural crop characteristics. Remote Sensing of Environment, 71: 158–182.

Thenkabail, P.S., E.A. Enclona, M.S. Ashton and B. Van Der Meer (2004) Accuracy assessments of hyperspectral waveband performance for vegetation analysis applications. Remote Sensing of Environment, 91: 354–376.

Tucker, C.J. (1980) A critical review of remote sensing and other methods for non-destructive estimation of standing crop biomass. Grass and Forage Science, 35: 177–182.

Vallentine, J.F. (2000) Grazing management, Elsevier.

Vanderbilt, V., L. Grant, L. Biehl and B. Robinson (1985) Specular, diffuse, and polarized light scattered by two wheat canopies. Applied Optics, 24: 2408–2418.

Vapnik, V. (2013) The nature of statistical learning theory, Springer Science & Business Media.

Wold, S., M. Sjöström and L. Eriksson (2001) PLS-regression: a basic tool of chemometrics. Chemometr. Intell. Lab., 58: 109–130.

Woolley, J.T. (1971) Reflectance and transmittance of light by leaves. Plant Physiology, 47: 656–662.

Yoshitoshi, R., N. Watanabe, T. Yasuda and. (2016) Methodology to predict the spatial distribution of cattle dung using manageable factors and a Bayesian approach. Agriculture, Ecosystems & Environment, 220: 135–141.

Zhao, D., P.J. Starks, M.A. Brown, W.A. Phillips and S.W. Coleman (2007) Assessment of forage biomass and quality parameters of bermudagrass using proximal sensing of pasture canopy reflectance. Grassland Science, 53: 39–49.

Intensive Livestock Farming on New Zealand Hill Country Farms Creates Critical Source Areas of Potential Pollution

Keith BETTERIDGE[1], Kensuke KAWAMURA[2], Des COSTALL[3], Siva GANESH[4], Dongwen LUO[4], John KOOLAARD[4] and Rena YOSHITOSHI[5]

[1]FarmSense, 72 Pahiatua St, Palmerston North, New Zealand
[2]Japan International Research Center for Agricultural Sciences (JIRCAS), Social Sciences Division, Tsukuba, Ibaraki, 305-8686, Japan
[3]28 Surrey Crescent, Palmerston North, New Zealand
[4]AgResearch Ltd, Private Bag 11008, Palmerston North, New Zealand
[5]Hiroshima University, Higashi-Hiroshima, Hiroshima, 739-0046, Japan

Corresponding Author: Keith Betteridge, keith@farmsense.co.nz

Keywords: urine sensor, cattle, sheep, predictive model, sustainable farming

Abstract

Nitrogen (N) from animal urine is a major potential water pollutant coming from grazed hill pastures in New Zealand. To ensure access to world markets, food must be produced sustainably. This research programme identifies reasons for and location of potential critical source areas (CSAs) of N which might lead to cost-effective, practical opportunities to mitigate N pollution. Urine sensors and GPS units for cows and ewes located urination events. Motion sensor data, a GIS and statistical models, made it possible to predict sheep resting and urination sites in response to variation in pasture mass and quality, slope, elevation and aspect. Aggregated urination events, or potential CSAs, were widespread at higher elevations within sheep paddocks. Because resting and urination zones were heterogeneously distributed, yet highly auto-correlated (r = 0.88), maps of resting areas alone predicted CSAs (R^2 = 0.82). Cattle resting and urination areas were more pronounced, with ~50% of urination events found in just 5-16% of paddock areas, generally in small, low-slope (≤12°) areas of steep paddocks and frequently near waterways. Models located cattle CSAs using GPS-resting and paddock contour data only. Greatest urinary N loads per cow urination occurred at night. Because these will mainly be excreted in campsites, >50% of daily urinary N will be excreted and leached from campsites. Farmers can probably access most of these small, low-slope areas, to target mitigation strategies to reduce N leaching. Contour maps alone might be sufficient to identify CSAs, while GPS tracking and mitigation records would prove resource consent compliance.

1. Introduction

Farmed hills have a high diversity of vegetation resulting from micro-environmental characteristics related to elevation, slope and aspect. Compared to low-slope land, soil on steep slopes are generally shallow, have a poor water holding capacity and can therefore generate greater overland flow that reduces soil fertility (Saggar et al., 1990; Lambert et al., 2000; Parfitt et al., 2009; Hoogendoorn et al., 2016). These factors affect plant species distribution and pasture growth production and, therefore, where animals prefer to graze (Rowarth et al., 1992; Lambert et al., 2000, 2003). Southern hemisphere south-facing slopes are cold and wet in winter and often have slow pasture growth rates compared to north-faces, but are warm and moist in summer, with high pasture growth rates. North-facing slopes are warmer in winter, but often hot and dry in summer. Summer-growing, highly nutritious legumes are sparse on north-facing slopes and these soils may be relatively more N deficient than on south faces. Without careful fence placement, stock transfer of plant nutrients from slopes to flatter areas and from cool to warmer areas (Saggar et al.,1990; Rowarth et al., 1992; Lambert et al., 2000) results in very high N leaching rates from flatter areas (Parfitt et al.,2009; Hoogendoorn et al., 2016).

McDowell & Srinivasan (2009) describe critical source areas (CSAs) for sediment, phosphate and faecal coliforms as zones in the landscape with excessive nutrient load that intersect an active hydrologic transport mechanism that poses a high risk for excessive pollutant export to surface waters. As urinary N is highly soluble it leaches to groundwater and is transported by sub-surface flow to streams and ponds (Ledgard 2001; White et al., 2001). Aggregation of excreta into CSAs in grazing systems is common in both hill country and lowland (White et al., 2001; Betteridge et al., 2012; Cai and Akiyama, 2016). Whereas 50-60% of excreted N is as urine, most excreted P is in faeces (Orr et al., 2012).To mitigate N pollution of water we must provide farmers with practical and affordable management tools to implement on their farm. This is challenging for farmers on steep hill country farms which can be hundreds to thousand hectares in size and which are extensively dissected by a mosaic of streams.

Satellite remote sensing is widely used to describe slope, aspect and elevation of landscapes and hyperspectral imaging is used to quantify biomass (BM; kg dry matter (DM)/ha) and nutrient content (e.g. Lilienthal et al., 2007; Kawamura and Akiyama, 2012). Defining why and where animals congregate (stockcamps), and the amount of excreta deposited in these camps is now possible using cheap GPS tracking devices on cattle and sheep (Betteridge et al., 2010a; Betteridge et al., 2012); and urine sensors for female cattle and sheep can count and log the number of urination events (Betteridge et al., 2010b; Betteridge et al., 2012; Benke et al., 2015). A new sensor estimates the amount of cows' urinary N excreted in each urination event (Betteridge et al., 2013b; Shepherd et al., 2016) which enables CSAs within grazed paddocks to be ranked for their relative risk to stream pollution. This is especially important with cattle-grazed pastures which leach twice as much nitrate-N/ha as sheep- and deer-grazed pastures, on an equivalent DM intake/ha basis (Hoogendoorn et al., 2011).

This review describes NZ hill country research linking animal behaviour to landscapes over short time scales, within 0.5 to 11 ha paddocks. Predictive models are described that enable farmers to map CSAs and strategically manage grazing to mitigate potential N pollution. The overarching goal is to enable sustainable intensification of food production in hill country (Roche et al., 2016).

2. Methods and Materials

Hand-held, GPS and hyper-spectral imaging devices were used to create digital elevation models (DEM) defining slope, aspect and elevation of grazed paddocks and maps of BM and pasture mineral mass variability as potential attractants to grazing sheep (Trial 1). At other sites, DEM and animal sensor data were the main inputs for model development.

2.1. Female sheep and cattle urine sensor *(Type I)*

This device, anchored in the vagina, detected each urination event by a change from ambient to body temperature as urine flowed over it (Fig.1a,b). Concurrent GPS tracking showed the location of each urination event (Betteridge et al., 2010a, b). These custom-made urine sensors and GPS units were cheap and easily fitted to animals. Animals could be left to graze in large steep or flat paddocks for the duration of the battery's power supply.

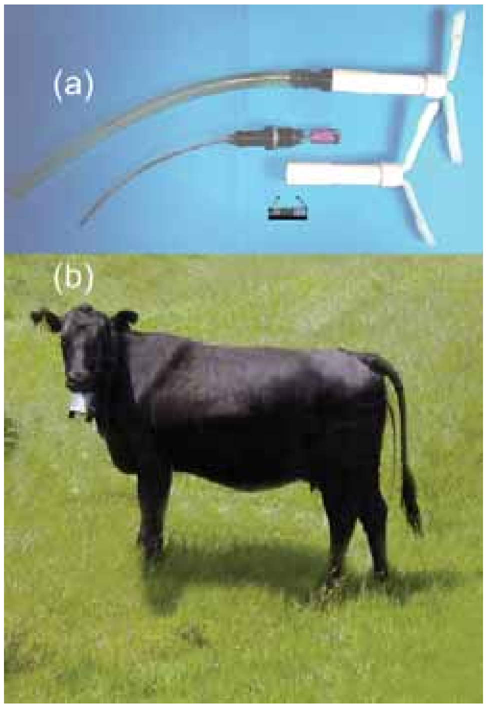

Fig.1. (a) Upper picture: is the complete sensor, with the white section inserted into the vagina; lower image shows sensor components (left to right: thermistor, circuitry and battery, waterproof pipe with soft silicon 'wings' to retain sensor within the vagina. Temperature is measuresd as mV.
(b) lower picture: sensor seen under cow's tail.

2.2. Female urine sensor *(Type II)*

This larger, heavier urine sensor was developed for cows to quantify urine volume and urinary N concentration [N] (Fig.2b; Betteridge et al., 2013b; Shepherd et al., 2016). Urine flow through the sensor (Fig.2a) creates a head of pressure which is continuously logged. Volume is determined by the area under the curve of an 'event duration v. pressure' graph. Urinary N concentration is estimated using a calibrated refractive index sensor (MISCO, Ohio, USA). The sensor, fixed to a cow cover (Fig.2b), is suspended under the tail, and the 'urine collector' is glued over the vulva to entrain urine into the sensor, without faecal contamination.

2.3. Research studies

To determine the number of urinations and their distribution within paddocks, grazing trials were conducted on farms near Lake Taupo and near Palmerston North, NZ. Four sites comprised flat and steep zones of 'browntop (*Agrostis capillaris*)/ ryegrass (*Lolium perenne*)/white clover (*Trifolium repens*)-based pastures. Monitored animals grazed within larger mobs of sheep or herds of cattle. Trial 5 was with dairy cows on flat land grazing ryegrass/ white clover pastures.

2.3.1 Trial 1 (Betteridge et al., 2008; Kawamura et al., 2009): A Geographic Information System (GIS) array of 10 m* 10 m cells overlying the 2.8 ha paddock contained DEM layers; BM and pasture quality; animal location (sum time spent in grid cells - T_{min}) and urination event (sum events in grid cells – U_{events}) data, to answer the question "is sheep urine distribution related to variation in pasture BM, masses N, P, K and S nutrients, slope, aspect or elevation?" A flock of 80, 3-year-old sheep grazed the paddock for 6 days in autumn, with 20 sheep fitted with a GPS and *Type I* urine sensor. Using a real-time kinematic GPS, point heights were streamed across the paddock. Hyper-spectral radiometer readings (400-2400 nm) were taken at 330 approximately equal spaced sites to determine pasture BM and pasture N, P K and S masses. Partial Least Square regression (PLS) full-spectrum analysis of pasture BM and quality spectra provided GIS polygon cell data for mapping each parameter's spatial variability. Geographically Weighted Regression (GWR) and Ordinary Least Squares (OLS) regression models were used to determine relationships between T_{min} and U_{events} in relation to pasture and DEM variables.

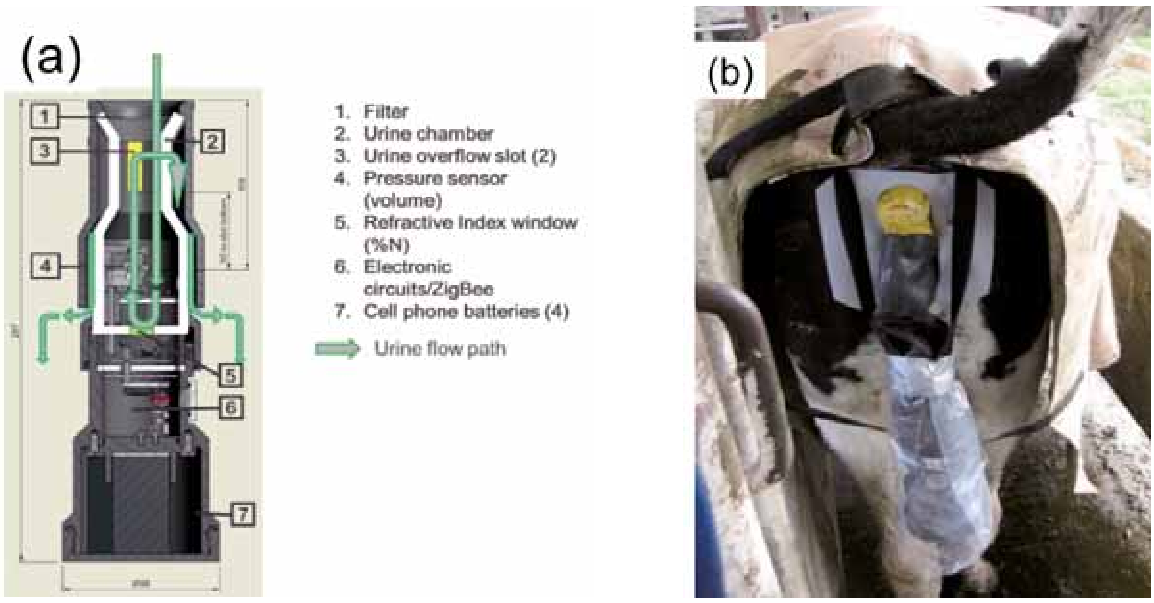

Fig.2. (a) *Type II* urine sensor showing the path of urine flow within chamber (2) and the refractive index sensor (5) that measures [N]. (b) Urine sensor attached to cow cover (black straps) with urine collector (yellow object) glued over the vulva. Grey patch assists retention of collector on the animal and polythene sleeve covers the sensor (a).

2.3.2. Trial 2 (Betteridge et al., 2010a)

Concurrently and just 1 km from Trial 1, within a 70 cow+calf herd, 20 beef cows were fitted with a *Type I* urine sensor and a GPS, grazed a steep 11 ha paddock over 7 days. The Paddock DEM was formed as in Trial 1. Pasture BM was not recorded, but was more than enough to provide the animals' needs. Grid cell T_{min} and U_{events} data were overlaid on the Paddock's DEM for determining spatial relationships.

2.3.3.1. Trial 3a (AgResearch's *Ballantrae* Research Farm near Palmerton North): Because farmers won't know animal movements or location of urine patches during a paddock's grazing, a surrogate parameter for predicting where they rest and urinate was needed. Twenty, 20-month-old female cattle were fitted with a *Type 1* urine sensor, motion sensor and GPS and monitored over 5 days while grazing a steep, 0.7 ha pasture. The paddock DEM was manually determined with a GPS. In the first instance, a *lying time* model (T_{min}) was created using independent variables, location (Eastings, Northings), slope, aspect and elevation (Betteridge et al., 2012).

2.3.3.2 Trial 3b A refined *resting* model (lying + standing time) based on a *threshold velocity of travel* within grid cells was developed with data from Trial 3a , and then tested with Trial 2 and Trial 3 data using just average velocity, and normalised location and DEM data. Normalisation makes resting time data compatible across paddocks. To confirm our concepts of CSAs in hill country, 83 soil samples were taken from GPS-located sites (campsites, shade trees, water trough, gateways and slopes) across 8 paddocks, with the Olsen P test used as the proxy for confirming nutrient transfer off slopes to flat areas (Betteridge et. al., 2013a).

2.3.4. Trial 4 (Massey University, Palmerston North)

Nine non-lactating cows were monitored for 11 days during winter while being offered a strip of new pasture each morning. Pasture was eaten in 2-3 hours. Cows were fitted with a GPS and *Type II* urine sensor and remained in the paddock once the strip of pasture had been eaten. Urinary nitrogen [N] and urine volume were used to calculate *N load* in each urination event (Betteridge et al., 2013b).

2.3.5. Trial 5 To estimate N leaching at the paddock scale, based on daily cattle data, Li et al. (2012a) used [N] and urine volume data of grazing beef steers (Betteridge et al., 1986) to develop a framework to assess the effects of varying cattle [N] and volume on N-leaching loss. Varying urine patch areas (urine spread) in relation to changing urine volumes were estimated and annual urinary N depositions were estimated. The model was driven by animal grazing days supported by pasture production (animal•days/ha/yr) and the average urine deposition events/animal/day. The N leaching from heterogeneous urine patches were scaled up to paddock level based on the frequency distribution of a range of urine patches. Measured [N] and volume data (Trial 4) were used within the framework to estimate the N leaching from Taupo pastures to demonstrate the effects of actual urine volumes and actual [N] on N leaching from grazed pastures.

3. Results and Discussion

3.1. Trial 1 (Betteridge et al., 2008)

Ordinary Least Square (OLS) regression models assume uniform variance amongst data across the paddock, but pasture BM and mineral mass data were highly heterogeneous and strongly auto-correlated. Therefore the GWR, using first derivative regression (FDR) data, gave better estimates of spatially varying parameters for BM, and mineral masses within the paddock. The R^2 value of 0.84 between actual and predicted BM, and similarly high R^2 values for N, P and S masses (kg/ha), were good for this difficult environment. Average number of sheep urination events was 20.6, but for sheep-within-days, ranged from 6 to 35. Sheep moved an average 2.6 km/day (1.3 – 4.1 km). Compared to low slope areas, sheep spent little time (T_{min}) on the top of hills or on steep sidelings. Low slope and flat areas were where their urination events (U_{events}) were concentrated (Fig.3). T_{min} and U_{events} were strongly correlated (r = 0.88), with each being less strongly correlated to *Elevation. Slope* was negatively correlated to T_{min} indicating sheep prefer to on flatter areas. Individual sheep were observed resting on small flat areas on hill slopes.

Although BM and mineral masses were strongly correlated, only S_{mass} was significantly correlated with T_{min} and U_{events} (r = –0.36 and –0.35 respectively). Using the backward stepwise selection method with OLS regression to predict spatial distribution of T_{min}

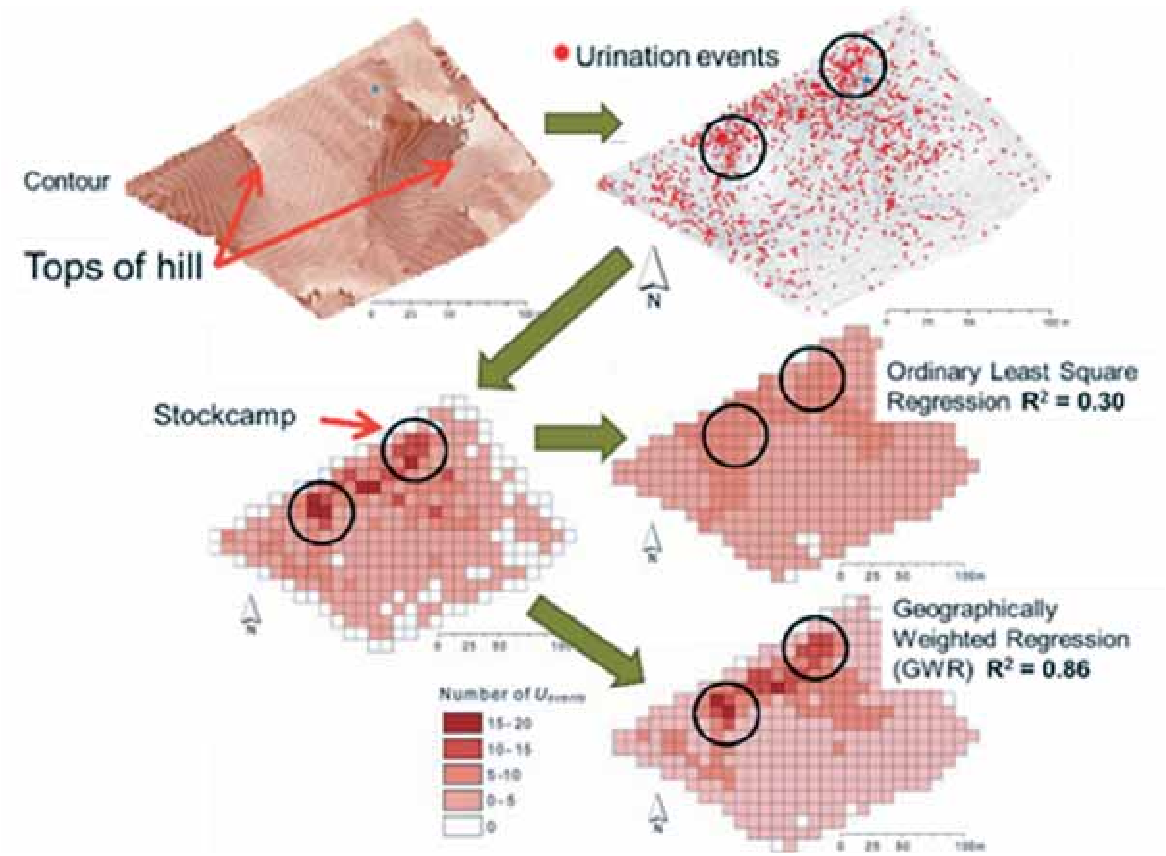

Fig.3. Inputs for predictive Ordinary Least Square and Geographically Weighted Regression models for mapping sheep urine distribution on a steep 2.86 ha paddock on a Taupo farm.

and U_{events} showed elevation, aspect and S_{mass} strongly influenced both T_{min} and U_{events} distribution, whereas only T_{min} was significantly influenced by Slope. The GWR predictive model with 8 independent variables gave much better goodness-of-fit (R^2 = 0.86 & 0.87) values for T_{min} and U_{events} respectively, than the OLS prediction (R^2 = 0.29 & 0.29). A simple regression to predict U_{events} based on T_{min} (R^2 = 0.75) showed T_{min} alone, might be sufficient to locate urine CSAs. The benefit of using GWR over OLS is seen in Fig. 3.

3.2. Trial 2 (Betteridge et al., 2010b)

Mean number of cow urination events was 12.3/day. The median slope of 10 m × 10 m polygon grid cells with at least one urination event recorded was 13.2° while the average slope of all these cells was 8.0°. This supports our contention that cattle camp on low slope areas in steep hill country.

To define where 9 cattle camped during the 4 grazing days (data of <24 h continuity were omitted) in this 11 ha paddock, a speed limit of ≤0.5m/sec within polygon cells with ≥2 urination events was set. The aggregation of cells in Fig.4a indicates cattle "camped' (rested and urinated) predominantly in low elevation, flat areas of the paddock and close to water troughs. The accumulated T_{min} regressed against U_{events} revealed a moderate correlation (r = 0.54). This correlation was lower for cattle than sheep (Trial 1) reflecting that; (1) there are five times more animals/ha in the sheep compared to cattle in the present trial, when stocked at the same stocking rate (sheep stock units (SU)/ha) and; (2) each sheep urinates ~20 times compared to cattle ~10 events/day. In this cattle paddock, 5% of the paddock contained 41%, and 10% of the paddock contained 61% of all urination events excreted during the grazing period.

As in Trial 1, it appears that a contour map could be used to predict the location of half of all cattle urine patches in steep hill country.

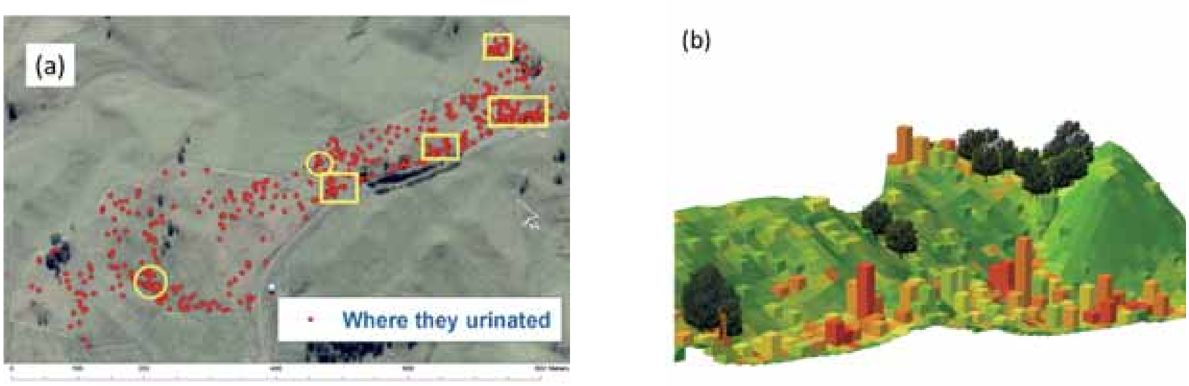

Fig.4. (a) Urine distribution by 9 cows grazing over 4 days in this steep, 11 ha paddock. Spots show urine patches; squares enclose groups of 10 m* 10 m cells in which velocity was <0.5 m/sec and in which there were ≥2 urination events; Circles show water troughs. (b) 3-D representation of the East end of Fig.4a. where histogram height within cells increases with number of urine patches and transistion from green to red shows increasing time spent in cells.

3.3.1. Trial 3a (Betteridge et al., 2012)

Cows urinated an average 9.0 events, lay down 45%, grazed 33%, stood 14% and walked 3% of the day. T_{min} was correlated with U_{events} (r = 0.64), with slope (–0.34) and dung deposition (0.49, manually mapped at the end of grazing). As in other trials, cattle lay down mainly in the only low-slope (0–12°) areas of this steep paddock. The correlation between T_{min} and U_{events} increased when *lying* and *standing* times were combined into a 'resting' variable (Fig. 5a). Near Neighbour Regression (kNNR), and the Generalised Additive Model (GAM), using associated grid values of Aspect, Slope, Elevation, Location (Eastings and Northings), gave the best prediction of T_{min} within 5 m* 5 m grid cells (Fig. 5b). Thus, knowledge of the paddock's contour features and Eastings and Northings could be used to predict resting places, and therefore, locate potential CSAs containing a high proportion of all urination events (and faeces) (White et al., 2001; Betteridge et al., 2012; Orr et al., 2012; Draganova et al., 2016).

3.3.2. Trial 3b (Betteridge et al., 2013a)

The *resting threshold model* determined the upper GPS threshold velocity of 0.11 m/sec, to represent when a cow was at rest (standing/lying). This approach dealt with 'misclassification' errors, random GPS errors, movement 'created' when disaggregating GPS travel speed between adjacent grid cells, and extended resting time intervals when no GPS readings were logged. Data normalisation was shown to be important when developing a generalised model for use over farms and regions.

It is clear in Fig.6 that the majority of cattle urine patch areas match the proxy resting time contours, especially where there were high densities of urine events (Betteridge et al., 2013a). In fact, of the grid cells with at least two urine events recorded, 86% also recorded lying events. When the urination events and lying times were accumulated over a 15 m × 15 m grid (formed using adjoining 5 m × 5 m cells), the correlation between urination events and lying time was r = 0.68. Of these larger cells with at least one urination event, 75% also recorded lying events. On a lowland dairy farm T_{min} was also correlated with U_{events} (r = 0.49; Draganova et al., 2016). Therefore, we suggest that the *resting threshold model* is a simple and efficient methodology for identifying potential CSAs within a paddock, based on animal GPS velocity and DEM data.

Soil Olsen phosphate data from obvious campsites, gateways, water troughs and under trees averaged 50.7 mg P/kg soil compared to just 13.4 mg P/kg soil on slope areas, confirmed the long-term accumulation and depletion of P transfer, via faeces, respectively. The strong relationship between faecal and urine deposition from this study and that reported by White et al. (2001) shows that areas around trees, gateways and water troughs within the paddock (White et al. , 2001; McDowell 2006; Draganova et al., 2016) need to be added to any CSA map based on DEM inputs alone.

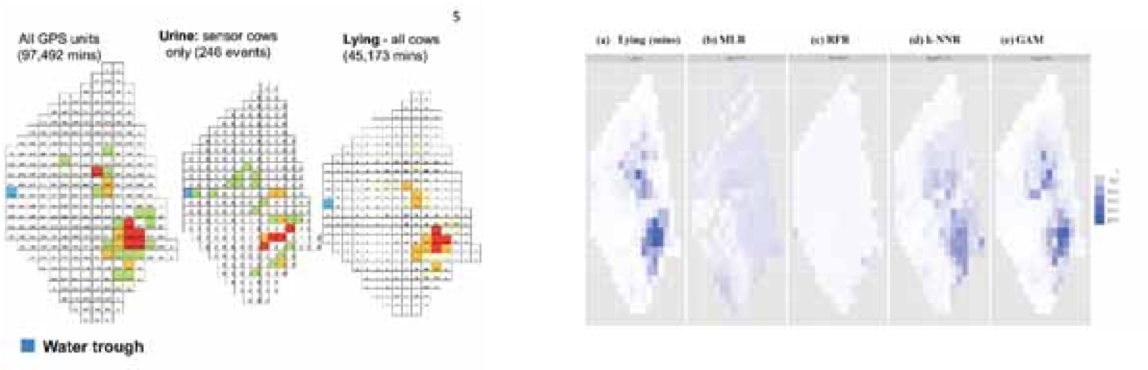

Fig.5. (a) Colour coding of 10 m * 10 m GIS grid cells show increasing values from green throughred of total minutes, urination events (urine sensor cows only) and lying time within cells of 20 cows over 5 days on this 0.7 ha steep hill paddock; (b) Actual lying time and predicted lying time based on Multiple Linear Regression(MLR), Random Forest Regression (RFR), Nearest Neighbour Regression (k-NNR), and Generalised Additive Model (GAM) models using a cross-validation test.

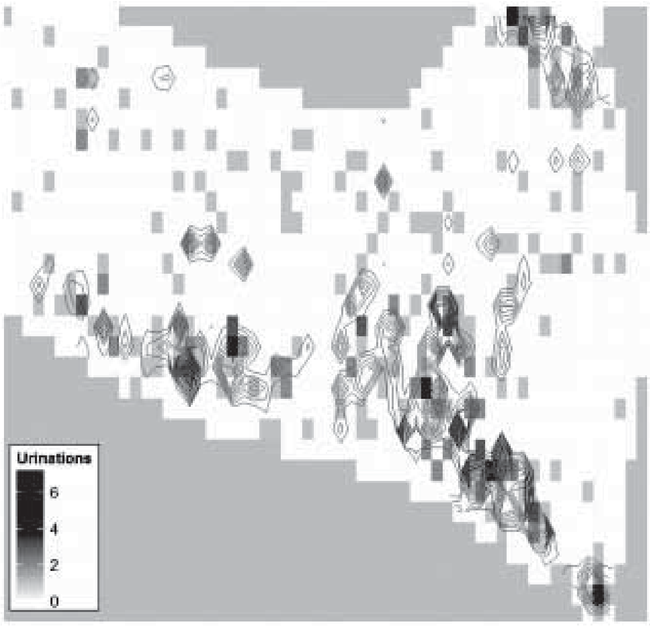

Fig.6. Contour lines created by the *Resting Threshold Model* showing where cows might camp in the small section of hill pasture shown in Fig.4b. Grey scale grid cells show the number of urination events known to have beenexcreted within grid cells.

3.4. Trial 4

Average volume of urination event was 2.1 L (SD 1.32) and ranged between 0.30 L and 7.83 L/event and average [N] was 0.95 g N/100 ml urine (SD 0.53), and ranged between 0.12 and 2.47 g N/100 ml (Betteridge et al., 2013b). Misselbrooke et al., (2013, 2016) used *Type II* sensors on grazing beef and dairy cattle in the UK and found similar urine characteristics.

At around 1-2 AM and at sunrise there was synchronised urination activity (Fig. 7). Most high N-load events occurred at night, though some smaller-load events sometimes followed large N-load events at these times. A high urination frequency occurred when cows were shifted to new pasture, but the N-loads were invariably small. Urinary N-loads/event became larger after grazing finished mid-morning. The majority of high N-load urination events in this trial occurred at night, which is probably true on all cattle farms. Hirata et al., (2011) also measured larger depositions of urine (and faeces) during the night than daytime, with frequency of deposition being higher in daytime. If so, such events in hill country would typically occur in stock camps on low-slope land predominantly at the bottom of the hill. Therefore, at the paddock scale, greatest N leaching

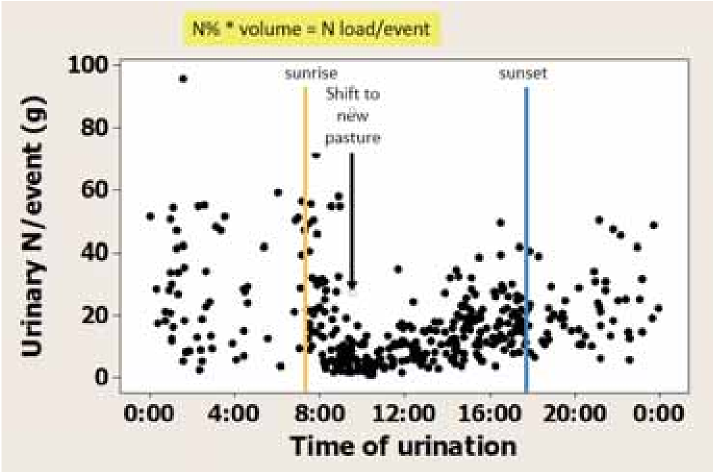

Fig.7 N-load of 9 non-lactating cows grazing a strip of fresh pasture each day over 11 days during winter. Cows wore a *Type II* urine sensor.

will occur under stock camps rather than from urine patches excreted during daytime. These daytime urination events will more likely be distributed across the paddock during grazing. Increasingly, lowland dairy farmers who are winter strip-grazing their cows will feed this pasture for 2-3 hr and then remove them to a holding pad. Our data suggests N loads will be small during this grazing period, only increasing once pasture is digested and surplus N excreted in urine once on the pad. Thus, the risk of N leaching from the grazed strip would be low, while the urinary N excreted on the holding pad could be managed in some manner to minimise N leaching (Di and Cameron 2016; Betteridge et al., 2013b).

3.5. Trial 5

A Case Study showed that if campsites received 30% of all urination events on just 3% of the paddock area, 37% more N would leach from "the whole paddock" compared to random application of the same amount of N (Li and Betteridge 2012b). Using model simulations of 2 years grazing followed by 2 years cropping, Hutchings et al. (2007) also reported greater rates of nitrate leaching when heterogeneity was included in the model, rather than the same amount of urinary N was applied uniformly over the paddock. Overlapping urine patches within the campsite was one main driver of increased leaching rate, as were urine patches with high N-loads. This is because N leaching increases exponentially as N load in the soil increases (Ledgard, 2001; McGechan and Topp, 2004; Shorten and Pleasants, 2007).

Many hill country farms have small areas on which they grow winter-fed crops and pastures. As cattle usually remain there for 2-3 months, these areas should be prioritised for implementing strategies to reduce N leaching (Betteridge et al., 2011).

To meet audit requirements of a Regional Authority, the farmer may need only print the farm's DEM showing zones of ≤12° slope, fence-lines, trees and streams and the GPS track of the farm vehicle used when applying the mitigation "tool".

If five sheep eat the same amount of N and excrete a similar amount of urinary as one cow, urinary N would be spread amongst 100 sheep urine patches compared to 10 urine patches for the cow (Betteridge 2010a). Thus the N load/urination event would be substantially higher in cattle, than in sheep, and be more clearly defined in cattle stockcamps than in the more diffuse, and smaller, sheep stockcamps (Betteridge et al., 2010a). This may explain why nearly double the amount of N leached from under cattle-grazed, than under sheep- or deer-grazed pastures stocked at a similar SU equivalence/ha (Hoogendoorn et al.2011). These findings make it clear that mitigation of N leaching on hill farms should be aimed at cattle-grazed paddocks.

Conclusions

New technologies have enabled the identification of urine distribution within grazed paddocks. Cattle create a greater N pollution threat to streams than sheep, so greatest emphasis on N leaching mitigation should be on cattle grazed pastures. It could be cost effective and practical for farmers to target these campsites with mitigation tools because only 5-15% of steep hill country might be used as campsites, yet contain ~50% of all urination events and more than 50% all excreted urinary N.

These zones could be determined from contour maps showing only the 12° isohyet to identify potential CSAs. Additional smaller CSAs under trees, around water troughs and gateways should also be targeted.

Many potential management strategies to decrease N loss from the urine patch are still at the proof of concept stage with few actually deployed on the farm (Monaghan et al., 2007; McDowell & Srinivasan 2009; Betteridge et al., 2011). Further research is required to integrate these into farm management systems (Selbie et al., 2015).

References

Augustine, D. J., D.G. Milchunas and J. D. Derner (2013) Spatial redistribution of nitrogen by cattle in semiarid rangeland. Rangeland Ecology and Management, 66:56–62.

Benke, K. K., F. Sheth, K. Betteridge, C. J. Pettit and J. P. Aurambout (2015) Application of geovisual analytics to modelling the movements of ruminants in the rural landscape using satellite tracking data. International Journal of Digital Earth, 8: 579–593.

Betteridge, K. and W. G. K. Andrewes (1986) Intake and excretion of nitrogen, potassium and phosphorus by grazing steers. Journal Agricultural Science, 106, 393–404.

Betteridge, K., K. Kawamura, D. Costall, M. Carter, C. Hoogendoorn, W. Griffiths and Y. Inoue (2008) Tools to determine impact of animal behaviour on nitrogen leaching and nitrous oxide emissions. Occasional Report No. 21, Proceedings of the workshop Carbon and Nutrient Management in Agriculture, L. D. Currie and L. J. Yates (Eds.), pp. 286–298.

Betteridge, K., D. Costall, S. Balladur, M. Upsdell and K. Umemura (2010a) Urine distribution and grazing behaviour of female sheep and cattle grazing a steep New Zealand hill pasture. Animal Production Science, 50: 624–629.

Betteridge, K., C. Hoogendoorn, D. Costall, M. Carter and W. Griffiths (2010b) Sensors for detecting and logging spatial distribution of urine from grazing cows and ewes. Computers and Electronics in Agriculture, 73: 66–73.

Betteridge, K., F. Li, D. Costall, A. Roberts, W. Catto, A. Richardson and J. Gates (2011) Targeting DCD at critical source areas as a nitrogen mitigation strategy for hill country farmers. In: Adding to the knowledge base for the nutrient manager, Currie, L. D. and C. Christensen (Eds.) , Massey University, Palmerston North, Fertilizer and Lime Research Centre. 11 pages.

Betteridge, K., S. Ganesh, D. Luo, K. Kawamura, D. Costall and R. Yoshitoshi (2012) A methodology for determining critical source areas of nitrogen in grazed hill pastures. In: Advanced Nutrient Management: Gains from the Past - Goals for the Future, Currie, L. D. and C. Christensen (Eds.), Massey University, Palmerston North, New Zealand, Fertilizer and Lime Research Centre. 10 pages.

Betteridge, K., D. Costall, D. Lou, S. Ganesh and H. Sun (2013a) Development of a critical source area prediction model of nitrogen leaching. In: Accurate and efficient use of nutrients on farms. L. D. Currie and C. L. Christensen (Eds.), Occasional Report No. 26. Fertilizer and Lime Research Centre, Massey University, Palmerston North, New Zealand. 8 pages.
http://flrc.massey.ac.nz/publications.html.

Betteridge, K., D. A. Costall, F.Y. Li, D. Luo and S. Ganesh (2013b) Why we need to know what and where cows are urinating – a urine sensor to improve nitrogen models. Proceedings of New Zealand Grassland Association, 75, pp.119–124.

Cai, Y. and H. Akiyama (2016) Nitrogen loss factors of nitrogen trace gas emissions and leaching from excreta patches in grassland ecosystems. A summary of available data. Science of the Total Environment, 572: 185–195.

Di, H. J. and K. C. Cameron (2016) Inhibition of nitrification to mitigate nitrate leaching and nitrous oxide emissions in grazed grassland: a review Journal of Soils and Sediments, 16: 1401–1420.

Draganova, I., I. Yule, M. Stevenson and K. Betteridge (2016) The effects of temporal and environmental factors on the urination behaviour of dairy cows using tracking and sensor technologies. Precision Agriculture, 17: 407–420.

Hoogendoorn, C. J., K. Betteridge, S.F. Ledgard, D.A. Costall, Z.A. Park and P.W. Theobald (2011)

Nitrogen leaching from sheep-, cattle- and deer-grazed pastures in the Lake Taupo catchment in New Zealand. Animal Production Science, 51(5): 416–425.

Hoogendoorn, C. J., P. C. D. Newton, B. P. Devantier, B. A. Rolle, P. W. Theobald and C. M. Lloyd-West (2016) Grazing intensity and micro-topographical effects on some nitrogen and carbon pools and fluxes in sheep-grazed hill country in New Zealand. Agriculture, Ecosystems and Environment, 217: 22–32.

Hirata, M., M. Higashiyama and N. Hasegawa (2011) Diurnal pattern of excretion in grazing cattle. Livestock Science, 142(1-3): 23–32.

Hutchings, N. J., J. E. Olesen, B. M. Petersen and J. Berntsen (2007) Modelling spatial heterogeneity in grazed grassland and its effects on nitrogen cycling and greenhouse gas emissions. Agriculture, Ecosystems and Environment, 121: 153–163.

Kawamura, K., K. Betteridge, I.D. Sanches, M.P. Tuohy, D. Costall and Y. Inoue, (2009) Field radiometer with canopy pasture probe as a potential tool to estimate and map pasture biomass and mineral components: a case study in the Lake Taupo catchment New Zealand Journal of Agricultural Research, 52: 417–434.

Kawamura, K., A. D. Mackay, M. P. Tuohy, K. Betteridge, I. D. Sanches and Y. Inoue (2011) Potential for spectral indices to remotely sense phosphorus and potassium content of legume-based pasture as a means of assessing soil phosphorus and potassium fertility status. International Journal of Remote Sensing, 32: 103–124.

Kawamura, K., Y. Sakuno, Y. Tanaka, H-J Lee, J. Lim, Y. Kurokawa and N. Watanabe (2011) Mapping herbage biomass and nitrogen status in an Italian ryegrass (*Lolium multiflorum* L.) field using a digital video camera with balloon system. Journal of Applied Remote Sensing, 5: 1–11.

Kawamura, K. and A. Akiyama (2012) Remote sensing for precision grassland management. Journal of the Remote Sensing Society of Japan, 32: 232–244. (in Japanese)

Lambert, M. G., Clark, D. A., Mackay, A. D., Costall and D. A. (2000) Effects of fertiliser application on nutrient status and organic matter content of hill soils. New Zealand Journal of Agricultural Science, 43: 127–138.

Lambert, M. G., A. D. Mackay, B. P. Devantier, D. B. McDougall, D. J. Barker and G. Park-Ng (2003) Redefining the production potential of hill pastures using fertiliser nitrogen. Proceedings of the New Zealand Grassland Association, 65: 35–40.

Ledgard, S.F. (2001) Nitrogen cycling in low input legume-based agriculture, with emphasis on legume/grass pastures Plant and Soil, 228: 43–59.

Li, F.Y., K. Betteridge, R. Cichota, C.J. Hoogendoorn and B.H. Jolly (2012a) Effects of nitrogen load variation in animal urination events on nitrogen leaching from grazing systems. Agricultural Ecosystems and Environment, 159: 81–89.

Li, F. and K. Betteridge (2012b) Assessing the effectiveness of applying nitrification inhibitor in stock-camps for mitigating nitrogen loss from grazed pastures. In Advanced Nutrient Management: Gains from the Past - Goals for the Future, L.D. Currie and C. L. Christensen (Eds), Occasional Report No. 25. Fertilizer and Lime Research Centre, Massey University, Palmerston North, New Zealand. 10 pages.

Lilienthal, H., A. Brauer, K. Betteridge and E. Schnug (2007) Monitoring land use change in the Lake Taupo catchment between 1975, 1990 and 2002 using satellite remote sensing data. Proceedings of the New Zealand Grassland Association, 69, pp.17–22.

McDowell, R. W. (2006) Phosphorus and sediment loss in a catchment with winter forage grazing off cropland by dairy cattle. Journal of Environmental Quality, 35(2): 575–583.

McDowell, R. W., M. S. Srinivasan (2009) Identifying critical source areas for water quality: 2. Validating the approach for phosphorus and sediment losses in grazed headwater catchments. Journal of Hydrology, 379: 68–80.

McGechan, M. B. and Topp, C. F. E., (2004) Modelling environmental impacts of deposition of excreted nitrogen by grazing dairy cows. Agricultural Ecosystems and Environment, 103: 140–164.

Misselbrook, T., C. Umstätter, C. A. Duthie, L. Nicoll and T. Waterhouse (2013) Automated monitoring of urination events from grazing beef cows. Precision Livestock Farming 2013 — Papers Presented at the 6th European Conference on Precision Livestock Farming, ECPLF 2013, Katholieke Universiteit Leuven, pp.211–216.

Misselbrook, T., H. Fleming, V. Camp, C. Umstätter, C. A. Duthie, L. Nicoll and T. Waterhouse (2016)

Automated monitoring of urination events from grazing cattle. Agriculture Ecosystems & Environment, 230: 191–198.

Monaghan, R. M., M. J. Hedley, H. J. Di, R. W. McDowell, K. C. Cameron and S.F. Ledgard (2007) Nutrient management in New Zealand pastures: recent developments and future issues. New Zealand Journal of Agricultural Research, 50: 181–201.

Orr, R. J., B. A. Griffith, R. A. Champion and J. E. Cook (2012) Defaecation and urination behaviour in beef cattle grazing semi-natural grassland. Applied Animal Behaviour Science, 139: 18–25.

Parfitt, R. L., A .D. Mackay, D. J. Ross and P. J. Budding (2009) Effects of soil fertility on leaching losses of N, P and C in hill country. New Zealand Journal of Agricultural Research, 52: 69–80.

Ravera, B. L., R. H. Bryant, K. C. Cameron, H. J. Di, G. R. Edwards and N. Smith (2015) Use of a urine meter to detect variation in urination behaviour of dairy cows on winter crops. Proceedings of the New Zealand Society of Animal Production 75 pp. 84–88.

Roche, J. R., S. F. Ledgard, M. S. Sprosen, S. B. Lindsey, J. W. Penno, B. Horan and K. A. Macdonald (2016) Increased stocking rate and associated strategic dry-off decision rules reduced the amount of nitrate-N leached under grazing. Journal of Dairy Science, 99 (7): 5916–5925.

Romera, A. J., G. Levy, P. C. Beukes, D. A. Clark and C. B. Glassey (2012) A urine patch framework to simulate nitrogen leaching on New Zealand dairy farms. Nutrient Cycling in Agroecosystems, 92(3): 329–346.

Rowarth, J. S., Gillingham, A. G., Tillman, R. W. and Syers, J. K. (1992): Effect of phosphate fertiliser addition and land slope on soil phosphate fractions. New Zealand Journal of Agricultural Research, 35: 321–327.

Saggar, S., A. D. Mackay, M. J. Hedley, M.G. Lambert and D.A. Clark (1990) A nutrient transfer model to explain the fate of phosphorus and sulphur in a grazed hill-country pasture. Agriculture Ecosystems & Environment, 30:
295–315.

Selbie, D. R., L. E. Buckthought and M. A. Shepherd (2015) The Challenge of the Urine Patch for Managing Nitrogen in Grazed Pasture Systems. Advances in Agronomy, 129: 229–292.

Shepherd, M. A., G. Brendon, G. Welten, D. Costall, P. G. P. Cosgrove, M. Pirie and K. Betteridge (2016) Evaluation of refractive index for measuring urinary nitrogen concentration in a sensor worn by grazing female cattle. New Zealand Journal of Agricultural Research:
DOI: 10.1080/00288233.2016.1237979.

Shorten, P. R. and A. B. Pleasants (2007) A stochastic model of urinary nitrogen and water flow in grassland soil in New Zealand, Agriculture, Ecosystems and Environment, 120 (2-4): 145–152.

White, S. L., R. E. Sheffield, S. P. Washburn, L. D. King and J. T. Green Jr (2001) Spatial and time distribution of dairy cattle excreta in an intensive pasture system. Journal of Environmental Quality, 30: 2180–2187.

The 14th International Symposium on Integrated Field Science

Development of Multi-functions of Plant Species Richness on Animal Production

Date: November 26-28, 2016

Venue: Graduate School of Agricultural Science (Amamiya Campus), Tohoku University, Sendai, Japan

Hosted by
Field Science Center, Graduate School of Agricultural Science, Tohoku University, Japan

Organizer:
Shin-ichiro Ogura

Professor
Laboratory of land Ecology,
Graduate School of Agricultural Science,
Tohoku University

Program

26 Saturday, November

16:00-18:00	Business meeting
18:00-20:00	Welcome banquet

27 Sunday, November

08:30–08:35	Opening Address: Makoto OSADA (Director of FSC, Tohoku University, Japan)
08:35–08:50	Scope of the Symposium: Shin-ichiro OGURA (Tohoku University, Japan)
08:50–10:30	**Session 1. Function of Plant Species Richness as Nutritional Source for Livestock**
1-1	Nutritional Characteristics of Forbs and Tree Leaves and Their Contribution to Animal Production in Species-rich Vegetation Shin-ichiro OGURA (Tohoku University, Japan) 08:50–09:20
1-2	Feeding Behavior and Animal Production of Korean Native Goats Grazed at Mountainous Pasture Sangho MOON (Konkuk University, Korea) 09:20–09:50
1-3	Effect of Plant Species Richness on Microbial Composition and Rumen Function Miwa NAKANO (NARO, Japan) 09:50–10:20
Discussion	
	Chairperson: Shin-ichiro OGURA (Tohoku University, Japan) 10:20–10:30
------ Tea Break -------	10:30–10:50
10:50–12:30	**Session 2. Behavioral Enrichment in Grazing Animals under Diverse Vegetation**
2-1	New Technologies for Monitoring of Foraging Behavior in Ruminants Masato YAYOTA (Gifu University, Japan) 10:50–11:20
2-2	Contribution of rearing at pasture on improvement of animal welfare in fattening pigs Akitsu TOZAWA (Tohoku University, Japan) 11:20–11:50
2-3	Effect of environmental enrichment on pituitary gland hormones and behavior of cattle Siyu CHEN (Chinese Academy of Agricultural Science, China) 11:50–12:20
Discussion	
	Chairperson: Masato YAYOTA (Gifu University, Japan) 12:20–12:30
12:30–13:30	Lunch
13:30–14:30	Poster session
14:30–15:40	**Session 3. Monitoring and Evaluation of Species Richness and Herbage Productivity in Pasture**
3-1	Spatial Heterogeneity in Grazing Pasture from Small UAV with Structure from Motion (SfM) Photogrammetry
3-2	Hyperspectral Assessment for Legume Contents and Forage Nutrient Status in Pasture Jihyun LIM (Hiroshima University) 14:30–15:00

3-3	Intensive Livestock Farming on New Zealand Hill Country Farms Creates Critical Source Areas of Potential Pollution Keith BETTERIDGE (FarmSense, New Zealand) 15:00–15:30
Discussion	
	Chairperson: Kensuke KAWAMURA (JIRCAS, Japan) 15:30–15:40
----- Tea Break -----	15:40–16:40
16:40–17:10	**Special Lecture**
	Byong-Tae JEON (Konkuk University, Korea)
17:10–17:20	Closing Remarks: Masato YAYOTA (Gifu University, Japan)
18:30–20:30	Banquet

28 Monday, November

	Excursion tour (Sendai city and Matsushima town)

Poster Session

1.	Kazuya DOI et al.	Gifu University	Effects of Stocking Rate on Forage Intake and Digestibility of Goats Grazing in an Abandoned Field in the Third Year
2.	Noriaki NAKAJIMA et al	Gifu University	Physiological and Immunological Differences in Cattle under Grazing or Confinement Condition
3.	Sae TAMIYA et al.	Gifu University	Effect of Plant Diversity on Ruminal Degradability of Goats Grazing in a Semi-natural Pasture
4.	Shinta TAKAMIZAWA et al.	Tohoku University	Does Species Richness of Diet Affect Ruminal Digestion Characteristics of Plants? —A Preliminary Study—
5.	Risa FUTAHASHI et al.	Tohoku University	Application of a Wearable Camera to Analyze Ingestive Behavior of Grazing Cattle
6.	Noritomo HATAKEYAMA et al.	Tohoku University	Foraging Behavior of Cattle in a Diverse, Mountainous Grazing Land: Bite Size Estimation of Plants by Hand-plucking Method
7.	K. SAITO et al.	Tohoku University	Uptake of Radioactive Cesium by Intestinal and Probiotic Bacteria
8.	Chinatsu YONEZAWA et al.	Tohoku University	Monitoring Temporal Vegetation Changes on Ungrazed Grassland by Satellite and Paramotor Remote Sensing
9.	Ayumi SADAIKE et al.	Tohoku University	Mix Cropping Trial of Determinate and Indeterminate Soybean Lines in Kawatabi Field Science Center

1-1. Nutritional Characteristics of Forbs and Tree Leaves and Their Contribution to Animal Production in Species-rich Vegetation

Shin-ichiro OGURA[1], Hayato MIZUNO[1], Shinta TAKAMIZAWA[1], Masato YAYOTA[2] and Kensuke KAWAMURA[3]

[1]Graduate School of Agricultural Science, Tohoku University, Japan
[2]Faculty of Applied Biological Science, Gifu University, Japan
[3]Japan International Research Center for Agricultural Sciences, Japan

Species-rich ecosystems provide us multi-functions such as supporting, provisioning, regulating and cultural services (Millennium Ecosystem Assessment 2005). In agricultural ecosystems, however, the effect of species richness on food production has not been fully understood. In species-rich grasslands, grazing animals encounter and consume a wide range of plant species, which likely affects the amount and proportion of dietary nutrients for grazing animals, because the nutrient composition of plants varies among species and access to a wider range of species provides animals a wider range of choices. To prove this hypothesis, grazing experiments were carried out by using beef cows in paddocks with different plant species count. Foraging behavior and botanical composition of diets in the cows, chemical composition of plants were measured. From these data, the concentration of nutrients in the diet and the amount nutrient intake per cow were estimated. The results showed that 1) the number of plant species foraged by grazing animals increased with increase of species richness of vegetation, 2) chemical composition of nutrients greatly differed among plant species; some minerals (Ca, Mg, Mn, Co and Se) concentration was higher in some forbs and tree leaves, 3) amino acid concentration was also high in some forbs, 4) higher amount of daily uptake was estimated in some minerals and amino acids in cows which grazed in species-rich paddocks than in grass dominant pasture. This study suggests that species rich vegetation including monocots, forbs and trees improve nutrient balance of grazing animals, due to the contribution of forbs and tree leaves which have high concentration of minerals and amino acids.

1-2. Seasonal Changes in Forage and Animal Productivity of Korean Native Goats Grazed at Different Forage Type

Se-Young JANG[1], Shin-ichiro OGURA[3], Kensuke KAWAMURA[4], Masato YAYOTA[5], Yeong-Sik YUN1, Hye-Jin SEONG[1], Sang-Woo KIM[2], Byong-Tae JEON[1] and Sang-Ho MOON[1]

[1]Division of Food Bio Science, Konkuk University, Chungju, 380-701, Korea
[2]Nation Institute of Animal Science, RDA, 590-832, Korea
[3]Graduate School of Agricultural Science, Tohoku University, Japan
[4]Japan International Research Center for Agricultural Sciences, Japan
[5]Faculty of Applied Biological Science, Gifu University, Japan

This study was conducted to determine grazing intensity of growing Korean native goats (*Capra hircus coreanae*) on mountainous pasture. It was carried out to obtain basic information for improvement of mountainous pasture management and establishing feeding system of Korean native goats. A total of 30 Korean goat were grouped by feeding system [a pasture grazing group (concentrate body weight 1%, treatment 1), a forage grazing group (concentrate body weight 1.5%, treatment 2) and a barn feeding group (TMR, treatment 3), n=10] to conduct study from May to October. Average daily gains (ADG) for the T1, T2 and T3 group were the highest in June for T1 (99.5±6.4 d/g), May for T2 (166.7±62.9 d/g) and May for T3 (206.7±7.1 d/g). The forage productivity of pasture was the largest from May to June (1718.7±207.5~1672.0±422.8 g/ha) but it was decreased in July (1356.0±103.8 g/ha) because of drought and summer depression. Grazing intensity was calculated by forage productivity and dry matter intake (DMI) and was the highest in May (65 head/ha, T1). Grazing intensity was calculated by forage productivity and DMI and was the highest in May for T1 (65 head/ha) and in May for T2 (58 head/ha). It is desirable that adequate grazing intensity was maintained by adjusting supplemental feed.

1-3. Effect of Plant-species Richness on Microbial Composition and Rumen Function

Miwa NAKANO

[1]Institute of Livestock and Grassland Science, NARO, Japan.

Plant diversity has been known to affect grassland ecosystem productivity and stability (Tilman *et al*. 2006), as well as the mineral balance in grazing cattle (Mizuno *et al*. 2011). However, there is little information on the relationship between plant diversity and livestock productivity traits such as rumen fermentation and digestion. Rumen bacteria play an essential role in the fermentation and digestion of cattle diet. In cattle fed fibrous diets, the rumen bacterial composition is found to be highly diverse (Petri *et al.* 2013), as plant-based fibers are rich in complex polysaccharides that enrich the microbial community (Krause *et al*. 2003). In microbiology, a high diverse microbial community is known to be able to respond quickly and flexibly to environmental changes (Miki 2011). Based on the above information, we proposed the following hypothesis: complex fibrous composition of plants and high fiber intake by cattle in native pastures with high plant diversity lead to high diversity in rumen bacteria, which ensures stable fermentation and digestion, as well as flexibility toward changes in feed. This year, we have investigated the rumen bacterial composition profile and rumen digestibility of grazing cows in two pastures with different plant species counts, by using molecular biology techniques and in vitro incubation, respectively. Stability will be evaluated in terms of changes in bacterial composition and digestibility after a change in the feeding regime from pasture to barn. I believe that understanding the relationship between plant diversity and livestock productivity will provide not only an evaluation of grassland ecosystem capability but also the primary knowledge for maintaining a stable rumen condition for livestock production.

2-1. New Technologies for Monitoring Foraging Behavior in Ruminants

Masato YAYOTA

Faculty of Applied Biological Sciences, Gifu University, Japan

Foraging behavior of grazing ruminants plays an important role in their production, as well as in maintaining plant diversity. Effects of plant species richness on animal production, health, and welfare are exerted only after animals have foraged the relevant plant species. Thus, monitoring foraging behavior is a key tool for understanding the underlying mechanism between plant species richness and animal production, health, and welfare. Efforts to monitor foraging behavior have, however, been confronted with methodological difficulty due to the complex and labor-intensive nature of the monitoring process. This difficulty increases when ruminants encounter more heterogeneous and diverse feeding environments, such as semi-natural or forest pastures, because they choose and ingest various plant species, which have different structures and morphologies. Recent developments in information and communication technology will drastically change this situation. Compact and wearable devices, such as acceleration sensors, can detect complex and fine animal movements precisely, and over a relatively long period. In this review, I firstly, and briefly, summarize the foraging process of grazing ruminants, which consists of prehending (biting) and processing (chewing) movements at a feeding station. Secondly, new technologies and a new methodology using an acceleration sensor and wearable camera to record and assess the foraging behavior of grazing ruminants are illustrated. Further, recent findings on foraging behavior in simple and diverse feeding environments, which were revealed by the new methods, are presented. Finally, the remaining issues regarding monitoring foraging behavior and several management implications of diverse feeding environments are discussed.

2-2. Contribution of Rearing at Pasture on Improvement of Animal Welfare in Fattening Pigs

Akitsu TOZAWA

Graduate School of Agricultural Science, Tohoku University, Japan

Rearing livestock at pasture is expected to improve animal welfare. On the other hand, it is thought to have possibility to loss physical health as the life in the outside environment is likely to contact with pathogens or being injured. To understand the contribution of rearing livestock at pasture on improvement of animal welfare, we examined behavior and health of fattening pigs by comparison to rearing outdoor pasturing system (OP) and indoor intensive system (IS).

From the behavior observations, the time budget of feeding concentration was at the same level (P = 0.69). OP pigs additionally expressed plant and soil eating, this resulted the total time budget of foraging became higher than IS pigs (P = 0.03). OP pigs spent exploring 3.7 times more (P = 0.03) and active 1.8 times more (P = 0.03) than IS pigs. OP pigs expressed play behavior (P = 0.05) as positive emotion and less disturbed behavior (P = 0.02) as negative emotion than IS pigs.

For physical health, we compared about incidence of pneumonia from *M. hyopneumoniae* (MPS score) and wounds on the body using the existing scoring method. Incidence of pneumonia was not affected by rearing system (P > 0.05). The score of wounds on the body of OP pigs was lower than that of IS pigs (P = 0.03). Rearing pigs at pasture have the possibility of improving physical health.

In conclusion, improvement of animal welfare for fattening pigs reared at pasture is provided by not only the opportunity to express normal behavior and become more active but also improving physical health.

2-3. Effect of Environmental Enrichment on Pituitary Gland Hormones and Behavior of Cattle

Siyu CHEN[1], Shin-ichiro OGURA[2] and Shusuke SATO[3]

[1]Department of Animal Nutrition and Feed Science, Chinese Academy of Agricultural Sciences, China
[2]Graduate School of Agricultural Science, Tohoku University, Japan
[3]Faculty of Life and Environmental Science, Teikyo University of Science, Japan

The pituitary gland hormone OT is a positive feedback hormone, the release of OT may cause behavior normalization, which can further accelerate OT secretion, and meanwhile may inhibit release of cortisol concentration. Here, we examined effect of environmental enrichment on OT and cortisol concentration, and investigated the relationship between pituitary gland hormone and social behavior. Material and methods: (1) Ten Japanese Black calves were divided into natural suckling (NS, n = 5) and bucket suckling (BS, n = 5) groups. NS calves were raised with dams, while BS calves were artificially raised from the age of 2 days, in single hutches. Blood samples were collected at the ages of 1 month and 1 week after weaning (2 months). Then 2 weeks after weaning each calf was put into the experimental pen with a novel decoy and each behavior was recorded for 20 min. (2) Six Japanese Black cows were brushed for 3 min a day for 10 days. Cows were then randomly assigned to two treatments of brushing or non-brushing for 3 min (-3 to 0 min). Time 0 was considered as the time brushing or no-brushing. Blood samples were collected at -6, 0, 3, 15, and 30 min. (3) Social behavior was investigated by continuous sampling of 16 Holstein lactating cows for 3 h; the total observation time was 18 h. Blood samples were collected three times for analyzing the OT and cortisol concentrations by using ELISA and EIA. Results: (1) OT concentrations of NS was significantly higher and the cortisol concentrations of NS was significantly lower than those of BS at 1 month ($P < 0.05$). OT concentration tended to correlate negatively with cortisol concentration ($P = 0.06$). Durations of contacts to the decoy and durations of the stay near the decoy were longer in NS calves than in BS calves (both $P < 0.05$). (2) Changes in OT concentrations with time (-6, 0, 3, 15, and 30 min) were significant different in brushing treatment but not in no-brushing treatment, whereas no significant change in cortisol concentration. OT concentrations at time 0 in the brushing treatment was significantly higher than those at times -6 ($P < 0.05$). (3) OT and cortisol concentrations, respectively, correlated positively with frequency of affiliative behavior ($r = 0.58$, $P < 0.05$) and frequency of receiving aggressive behavior ($r = 0.63$, $P < 0.01$). Thus, these two environmental enrichments, natural suckling and brushing were identified to enhance serum OT concentration in cattle. Futhermore, OT may encourage affiliative behavior and attenuate cortisol release.

3-1. Spatial Heterogeneity in Grazing Pasture from Small Unmanned Aerial Vehicle (sUAV) with Structure from Motion (SfM) Photogrammetry

Kensuke KAWAMURA[1], Jihyun LIM[2], Masato YAYOTA[3], Shin-ichiro OGURA[4], Se-Young JANG[5] and Sang-Ho MOON[5]

[1]Japan International Research Center for Agricultural Sciences, Japan
[2]Global Career Design Center, Hiroshima University, Japan
[3]Faculty of Applied Biological Science, Gifu University, Japan
[4]Graduate School of Agricultural Science, Tohoku University, Japan
[5]College of Biomedical and Health Sciences, Konkuk University, Korea

Site-specific management strategies in grazing ecosystem increase management efficiency. Due to complex interrelationship among soil-plant-animal-environment in grazing ecosystem, site-specific grazing management needs high measurement density to reflect their spatial patterns within the field. Recently, small unmanned aerial vehicles (sUAV) have been introduced into agricultural research. Images captured by sUAV are shown to be a potential alternative given their low cost of operation in pasture monitoring with high spatial and temporal resolution, and their high flexibility in image acquisition programming. Moreover, current developments in photogrammetric algorithms are specifically adapted to the needs of UAV imagery. Our goal is to clarify the interrelationship between plant-animal in grazed pasture for making precision grazing management. In this paper, we reviewed current developments of sUAV and photogrammetric algorithms, and their applications for pasture managements using our results mainly obtained at Hiroshima University farm in two grazing season of 2014-2015. The results include; (1) seasonal changes in floristic composition and nutritive status for dominant species, (2) spatial distribution of beef cattle with GPS collar attached, (3) application of sUAV to estimate vegetation coverage, herbage biomass and cow's dung detection, and (4) spatial relationship between plant and grazing behavior.

3-2. Hyperspectral Assessment for Legume Contents and Forage Nutrient Status in Pasture

Jihyun LIM[1], Kensuke KAWAMURA[2], Rena YOSHITOSHI[1], Masato YAYOTA[3] and Shin-ichiro OGURA[4]

[1]Global Career Design Center, Hiroshima University, Japan
[2]Japan International Research Center for Agricultural Sciences, Japan
[3]Faculty of Applied Biological Science, Gifu University, Japan
[4]Graduate School of Agricultural Science, Tohoku University, Japan

The spectral assessment of pasture biomass and nutrient status is influenced by floristic composition. The accurate estimation of the nutrient status in a pasture throughout the growing season is challenging and a critical step to establish a site-specific management strategy for the improvement of productivity and profitability as well as the mitigation of the environmental impact. Remote sensing technologies have been widely applied to vegetation surveys because they can quickly retrieve the *in situ* biophysical and biochemical information of a field. Recent advances in sensing technologies, especially in a hyperspectral sensor system that has a higher spectral resolution of less than 10-nm bandwidth, have significantly improved predictive ability for the estimated biomass quantification in comparison with the conventional broad-band sensor system. Not only the biomass quantification but also other information about the pasture, such as forage nutrient content and the floristic composition, can also be estimated using its abundant spectral information, which is difficult to achieve on a broad-band sensor system. In this mini-review, we discuss the use of hyperspectral assessment to estimate the forage parameters of a pasture. Recent improvements in the analysis methodology of hyperspectral data have been reviewed and include (i) a univariate statistical approach based on narrow-band vegetation indices, (ii) multivariate statistical approaches, especially using partial least squares (PLS) regression, (iii) waveband selection to enhance the predictive performance of PLS regression, and (iv) the spatial interpolation of predicted values from ground-based hyperspectral measurements.

3-3. Intensive Livestock Farming on New Zealand Hill Country Farms Creates Critical Source Areas of Potential Pollution

Keith BETTERIDGE[1], Kensuke KAWAMURA[2], Des COSTALL[3], Siva GANESH[4], Dongwen LUO[4], John KOOLAARD[4] and Rena YOSHITOSHI[5]

[1]FarmSense, 72 Pahiatua St, Palmerston North, New Zealand
[2]Japan International Research Center for Agricultural Sciences (JIRCAS), Social Sciences Division, Tsukuba, Ibaraki, 305-8686, Japan
[3]28 Surrey Crescent, Palmerston North, New Zealand
[4]AgResearch Ltd, Private Bag 11008, Palmerston North, New Zealand
[5]Hiroshima University, Higashi-Hiroshima, Hiroshima, 739-0046, Japan

Nitrogen (N) from animal urine is a major potential water pollutant coming from grazed hill pastures in New Zealand. To ensure access to world markets, food must be produced sustainably. This research programme identifies reasons for and location of potential critical source areas (CSAs) which might lead to cost-effective, practical opportunities to mitigate N pollution. Urine sensors and GPS units for cows and ewes located urination events. Motion sensor data, a GIS and statistical models, made it possible to predict sheep resting and urination sites in response to variation in pasture mass and quality, slope, elevation and aspect. Aggregated urination events, or potential CSAs, were widespread at higher elevations within sheep paddocks. Because resting and urination zones were heterogeneously distributed, yet highly auto-correlated ($r = 0.88$), maps of resting areas alone predicted CSAs ($R^2 = 0.82$). Cattle resting and urination areas were more pronounced, with 50% of urination events found in just 5-16% of paddock areas, generally in small, low-slope (≤12°) areas of steep paddocks and frequently near waterways. Models located cattle CSAs using GPS resting and contour data only. Greatest urinary N loads per cow urination occurred at night. Because these will mainly be excreted in campsites, >50% of daily urinary N will be excreted and leached from campsites. Farmers can probably access most of these small, low-slope areas, to target mitigation strategies to reduce N leaching. Possibly contour maps alone might be sufficient to identify CSAs, while GPS tracking and mitigation records would prove resource consent compliance.

From 2017 to 2047

Byong-Tae JEON

Division of Food Bio Science, Konkuk University, Korea

I will retire and become an honorable professor next year. From 2017 to 2047 which is 30years from my retirement, it might be so hard to pick one among so many fun things to do. I would like to learn how to play guitar, to learn Chinese, and to learn martial arts in China. Reading books for being an author seems fun too. However, I might end up with following things than mentioned above.

First: The restoration campaign for wild deer. The annual fallen velvet antlers are great source of food for other animals. They are also great health food for human beings. In Korea, wild deer was extinguished at the end of 1500's. The reason why the god created deer and spread them all around the world is to sustain ecosystem. Because of extinction of wild deer, in Korea, the carnivores were extinguished and the soil is not fertile enough. In order to return to the normal ecosystem, to restore wild deer in Korea is my first task.

Second: the establishment of a university. Recently, the happiness of human being is concerned due to the following factors: the unstable climate environment and the advent of incurable diseases. Running a university for research about the happiness of mankind is my dream. Whether or not science will lead to human happiness is one of my topics.

To accomplish these two tasks, the cornerstone is to run my health food company successfully. Please be participative by purchasing the products.

Effects of Stocking Rate on Forage Intake and Digestibility of Goats Grazing in an Abandoned Field in the Third Year

Kazuya DOI[1], Sae TAMIYA[2] and Masato YAYOTA[2]

[1]The United Graduate School of Agricultural Sciences, Gifu University, Japan
[2]Faculty of Applied Biological Sciences, Gifu University, Japan

The objective of the present study was to evaluate the long-term effect of stocking rate on forage intake and digestibility of goats grazing in an abandoned field. In the present study, the results in the third year were presented. This experiment was conducted in an abandoned field (0.8 ha) in Minokamo City, Gifu, Japan, in 2015. The field was divided into two paddocks according to stocking rate (30 goats ha^{-1} and 14 goats ha^{-1}). Nine and seven goats grazed in the paddocks from June to October or November for three years. Three experimental periods of 14 days each were set during the grazing season. Plant biomass, forage intake, digestibility, body weight, and several serum parameters were measured in May (spring), July (summer), and October (autumn). Forage intake and digestibility of the goats were estimated using the *n*-alkane method. Plant biomass linearly decreased through the grazing season. Stocking rate did not affect dry matter (DM) intake and digestibility ($P>0.05$) of the goats, whereas DM digestibility tended to be lower in summer than in spring and autumn ($P=0.09$, quadratic) in both treatments. Despite the stocking rate, serum glucose concentration in the goats was lower than the critical value (41.1 $mg \cdot dL^{-1}$) in summer, and serum urea nitrogen concentration linearly increased with the season ($P<0.05$). However, body weight of the goats did not decrease with the season. These results suggest that goats grazing in an abandoned field can maintain their performance regardless of the stocking rate even in the third year.

Physiological and Immunological Differences in Cattle under Grazing or Confinement Condition

Noriaki NAKAJIMA[1], Kazuya DOI[1], Sae TAMIYA[2] and Masato YAYOTA[2]

[1]The United Graduate School of Agricultural Science, Gifu University, Japan
[2]Faculty of Applied Biological Sciences, Gifu University, Japan

Grazing is assumed to have positive effects on the animal welfare of livestock; however, the underlying mechanism is still unclear. Physiological and immunological states reflect the level of animal welfare. However, little information is available for evaluating the physiological and immunological states of grazing animals to improve their welfare. The objective of the present study was to determine the physiological and immunological differences in cattle under grazing or confinement by using blood parameters. Ten Japanese black cows (337±50 kg) were used in this experiment. All cows were kept confined for two weeks. Then, five of the ten cows were grazed for two months on a 1.8 ha field composed of sown pasture and forest land, and the remaining cows were fed under confinement. After two months, all cows were kept confined for two weeks. Blood samples were collected from the cows every two weeks, throughout the experiment. The number of blood cells was estimated by Celltac α (Nihon Kohden Co., Ltd.). The type of white blood cells was identified by May-Grunwald and Giemsa staining. Plasma biochemical parameters were analyzed using a dry-chemistry method (Fujifilm Co., Ltd.). The number of red blood cells in grazing cows was lower than that in confined cows. The number of neutrophils, monocytes, and platelets in grazing cows was higher than that in confined cows. Plasma phosphate and blood urea nitrogen concentrations fluctuated during grazing. These results suggest that phagocytosis and clotting ability improved under grazing condition.

Effect of Plant Diversity on Ruminal Degradability of Goats Grazing in a Semi-natural Pasture

Sae TAMIYA[1], Kazuya DOI[2], Noriaki NAKAJIMA[2], Shin-ichiro OGURA[3] and Masato YAYOTA[1]

[1]Faculty of Applied Biological Sciences, Gifu University, Japan
[2]United Graduate School of Agricultural Sciences, Gifu University, Japan
[3]Graduate School of Agricultural Science, Tohoku University, Japan

The objective of this study was to clarify the effect of plant diversity on ruminal degradability of goats grazing in a semi-natural pasture. The experiment was conducted in July and August 2016 in a semi-natural pasture in Minokamo City, Gifu, Japan. The pasture was divided into two paddocks according to stocking rate: high (HS: 30 goats ha^{-1}) and low (LS: 14 goats ha^{-1}). Direct and continuous observations of bites taken were used to record ingested plants by four grazing goats in each paddock. Five dominant plants in the ingesta—fresh materials of *Poa* spp., *Erigeron annuus, Paederia foetida, Phyllostachys edulis,* and white clover and two common grasses—fresh Italian ryegrass and Timothy hay were used for evaluating *in vitro* ruminal degradability. Rumen fluids were collected from three goats in each paddock by using a stomach tube. The plants were incubated by the batch culture method. The grazing goats in HS ingested 48 and 47 species in July and August, respectively, whereas the goats in LS ingested 63 and 57 species in July and August, respectively. The goats in LS ate more woody plants and less herbaceous plants than did the goats in HS. *In vitro* degradability of Italian ryegrass and *Phyllostachys edulis* was higher in HS than in LS (P<0.05), whereas no difference was detected in other species between the stocking rates. The degradability of the two common grasses was higher in July than in August; however, no seasonal effect was detected in the degradability of the five dominant species.

Does Species Richness of Diet Affect Ruminal Digestion Characteristics of Plants? —A Preliminary Study—

Shinta TAKAMIZAWA, Tetsuro SHISHIDO and Shin-ichiro OGURA

Graduate School of Agricultural Science, Tohoku University, Japan

In this study, *in vitro* digestion experiment was conducted to examine the effect of plant species richness in diet on ruminal digestion of plants in grazing cattle. One grass (*Dactylis glomerata*), two forbs (*Trifolium repens* and *Rumex acetosella*) and one tree species (*Acer rufinerve*) were collected from a grazing paddock of Field Science Center, Tohoku University, Japan, in mid-summer. These samples were air-dried and grounded (0.5 mm screen). Ruminal fluid was collected by using oral catheter from cows grazed on a mountainous area (pasture + forest; PF) and an orchard grass sown pasture (SP), and filtrated by using duplicate gauze. These rumen fluid samples were used as inoculum of digestion experiment after dilution with McDougall artificial saliva (1:4) and saturated with CO_2. The plant samples (0.5 g) and the rumen inoculum (50 mL) were incubated for 0, 24, 48 h at 39°C. Residue was collected by filter paper and dried at 105°C for 16 h to determine dry matter digestibility (DMD). The effect of plant species, ruminal fluid and these interaction were all significant (P<0.05) after 24–48 h incubation. In *A. rufinerve*, DMD was significantly lower than in other plant species for all incubation time (P<0.01). DMD of forbs was significantly different between ruminal fluid treatment (P<0.05); DMD of *R. acetosella* was PF>SP after 24 h incubation, and that of *T. repens* was SP>PF after 24 h but PF>SP after 48 h incubation. In contrast, there was no significant difference in *D. glomerata*. The results suggest that species richness of diet affect ruminal digestibility of plants.

Application of a Wearable Camera to Analyze Ingestive Behavior of Grazing Cattle

Risa FUTAHASHI[1], Masato YAYOTA[2], Noritomo HATAKEYAMA[1], Tetsuro SHISHIDO[1] and Shin-ichiro OGURA[1]

[1]Graduate School of Agricultural Science, Tohoku University, Japan
[2]Faculty of Applied Biological Sciences, Gifu University, Japan

In this study, we monitored ingestive behavior of grazing cattle by using a wearable camera, to evaluate usefulness of the tool for continuous identification of plant species ingested. Eight beef cows (mean body weight was 520±104 kg/head) grazed in a pasture-forest combined area (more than 70 plant species existed) in early summer. Two cows were chosen as focal animals, and a wearable camera with microphone (60 mm×94 mm×27 mm, 185 g, HX-A500, Panasonic, Osaka, Japan) was attached onto the front strap of each cow, approximately about 20 cm apart from the right corner of mouth. Ingestive behavior was recorded 90 minutes during a grazing bout in the afternoon. A direct observation of the cows was also carried out simultaneously to compare preciseness of the camera. By the measurements, 27–60 minutes of ingestive behavior were recorded; 213–485 bites were observed and 15 plant species were identified by a direct observation and the camera in combination. Among these data, the wearable camera and direct observation identified 205–469 bites (96.2–96.7% of total bites) and 188–417 bites (86.0–88.3% of total bites), respectively. The wearable camera missed 3.3–3.8% of total bite events mainly due to access from a blind angle to the plants. On the other hand, direct observation missed 11.7–14.0% of total bites, mainly due to rapid movement of the animals (4.9–5.2%) and a small amount of bite size (2.8–3.7%) in addition to the access from a blind angle (3.8–5.4%). These results suggest that the wearable camera is useful to record ingestive behavior of grazing cattle more precisely than direct observation. However, it is necessary to eliminate blind spots to improve the accuracy of recording with the camera.

Foraging Behavior of Cattle in a Diverse, Mountainous Grazing Land: Bite Size Estimation of Plants by Hand-plucking Method

Noritomo HATAKEYAMA[1], Risa FUTAHASHI[2], Tetsuro SHISHIDO[2] and Shin-ichiro OGURA[2]

[1]Faculty of Agriculture, Tohoku University, Japan
[2]Graduate School of Agricultural Science, Tohoku University, Japan

Hand-plucking method was conducted in a diverse, mountainous grazing area, to clarify whether bite size of cattle is affected by plant species and foraging height. The data was collected at a pasture-forest combining grazing area in the Field Science Center, Tohoku University, north-east district in Japan, in early summer and early autumn. Biting frequency and foraging manner of individual plant species were estimated by visual observation of focal animals. Foraging heights were also recorded simultaneously. Based on these data, 11 major plant species (six herbaceous plants, four trees and one vine) were chosen and plant samples were collected by hand-plucking method mimicking foraging manner of the animals by four persons. For trees and vine, samplings were done from three different layers (upper: 120-180 cm, middle: 60-120 cm, lower: 0-60 cm) which were set based on the position of animal's head. There was a significant difference in bite size among plant species; *i.e.*, bite size of *Acer rufinerve* and *Carex albata* were significantly higher than other plants (P<0.05). Bite size was also higher in summer (0.31 g DM/bite) than in autumn (0.19 g DM/bite) (P<0.001), but there was a significant interaction between plant species and season (P<0.01). For trees and vine, bite size in upper layer showed a tendency to be higher than in middle and lower layer (P=0.071). *In Viburnum dilatatum,* a significant higher bite size was observed in upper layer than in middle and lower layers. These results show that greater bite size can be obtained by foraging of tree species, when defoliation from upper layers is beneficial for the animals to increase bite efficiency throughout the seasons.

Uptake of Radioactive Cesium by Intestinal and Probiotic Bacteria

K. SAITO[1], K. KURODA[1], T. SEKINE[1], M. FUKUMOTO[1], Y. KINO[1], T. FUKUDA[1],H. YAMASHIRO[2], H. SHINODA[1], H. ISOGAI[4], J. KOBAYASHI[5], J. NISHIMURA[3],Y. ABE[6], N. NISHIDA[7], M. KOUNO[7] and E. ISOGAI[1]

[1]Tohoku University, Sendai, Miyagi, Japan
[2]Niigata University, Niigata, Japan
[3]Hachinohe Institute of Technology, Hachinohe, Aomori, Japan
[4]Sapporo Medical University, Sapporo, Hokkaido, Japan
[5]Miyagi University, Sendai, Miyagi, Japan
[6]Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido, Japan
[7]Morishita Jintan Co., Ltd., Japan

Large amounts of radioactive materials emitted by the Fukushima Daiichi nuclear power plant (FNPP) accident in March 2011 have caused a heavy environmental pollution. Especially, food contamination by radioactive cesium (Cs) made headlines throughout the world. Germanium gamma-ray spectrometry detected peaks from ^{134}Cs and ^{137}Cs in the grasses. Stray cattle ate them at evacuation zone within 20km of FNPP. To demonstrate the decontamination mechanism from human and animals, we conducted the uptake of radioactive Cs by intestinal and probiotics bacteria.

We prepared brain-heart infusion (BHI) medium containing radioactive Cs, and inoculated *Bifidobacterium longum*, *Clostridium perfringens*, *Clostridium ramosum*, *Bacteroides fragilis*, and *Bacteroides vulgates*. Similarly, *B. longum*, *B. breve*, *Lactobacillus gasseri*, *Lactobacillus casei* and *Lactobacillus delbrueckii* subsp. *bulgaricus* were inoculated onto the de Man, Rogosa, and Sharpe (MRS) medium containing radioactive Cs. After 48 hours, radioactive Cs uptake of bacterium was examined. Uptake rate ranged from 37.8 to 81.2 % in *Bifidobacterium*, *Clostridium* and *Bacteroides*, by contrast *Lactobacillus* only 2-3%. These results indicate that intestinal bacteria could take in radioactive Cs as efficient scavengers. On the other hand, *Lactobacillus* did not showed uptake efficiently. We presumed potassium (K) in medium inhibited uptake of Cs. So, we investigated the K concentration of these mediums. The K concentration of BHI and MRS medium was 0.02% and 0.14%, respectively. K concentration of MRS is significantly higher than BHI. The result indicated that K inhibited bacterium uptake of radioactive Cs. As a future activity, we will carry out experiments on medium of low K concentration.

Monitoring Temporal Vegetation Changes on Ungrazed Grassland by Satellite and Paramotor Remote Sensing

Chinatsu YONEZAWA, Shin-ichiro OGURA and Masanori SAITO

Graduate School of Agricultural Science, Tohoku University, Japan

Integrated Terrestrial Field Station affiliated with Graduate School of Agricultural Science, Tohoku University, called as Kawatabi Field Science Center (FSC) is located in a hot spot area polluted by Fukushima Daiichi nuclear disaster triggered by the 2011 Tohoku earthquake. Large grazing pastures are included in FSC and cattle were grazed before the pollution. After the pollution, the cattle grazing has been voluntary restrained.

In this study, we compared time-series of remote sensing images for the grass land area on FSC to evaluate the effect of the ungrazing by the disaster on vegetation. The images obtained from high resolution satellites, IKONOS in July, 2008, WorldVie-2 in November, 2010, October, 2014 and Quickbird in July, 2012 were used. The spatial resolution of panchromatic images IKONOS, WorldView-2 and Quickbird is 0.8, 0.5 and 0.6m respectively. Pan-sharpened dataset was created by Gram-Schmidt Spectral Sharpening method. The images obtained from paramotor in October, 2013 and September, 2015 were also used for the comparison. The spatial resolution was approximately 0.1 m and 0.19 m, respectively.

Though the difference of observation season is an element of consideration for the image interpretation, temporal vegetation change is recognized. Increase in the number of grass plant or shrub blocks is estimated in all parts of the grass land. Especially, in the comparison of the images obtained from paramotor, the increase of around 1–2 m forms is recognized. These forms correspond to large grass plants such as Japanese silver grass and round-leaved dock.

Mix Cropping Trial of Determinate and Indeterminate Soybean Lines in Kawatabi Field Science Center

Ayumi SADAIKE[1], Momoko OGAWA[1], Koki HOMMA[1,2], Toyoaki ITO[1,2], Takayuki NAKAJIMA[1], Yoshihisa SUYAMA[1,2], Shinji IIZUKA[3], Isao KARUBE[4], Yoshiaki MOTOYAMA[5] and Shin KATO[6]

[1] Graduate School of Agricultural Science, Tohoku University, Japan
[2]Applied Biodiversity Center, Graduate School of Agricultural Science, Tohoku University, Japan
[3]Hitachi Solutions East Japan, Ltd., Japan
[4]Hitachi, Ltd., Japan
[5]Office of Innovation and Business, Tohoku University, Japan
[6]Tohoku Agricultural Research Center, National Agriculture and Food Research Organization, Japan

Mix cropping of different cultivars is recently attracted due to its higher productivity and stability. This study focuses on mix cropping of determinate and indeterminate soybean cultivars.

Determinate soybean cultivars are commonly planted in Japan while indeterminate ones are planted in Midwest USA and discussed to be introduced in Japan. However, several-year trials showed no yield advantage of indeterminate soybean in the experimental field in Amamiya campus of Tohoku University. The indeterminate soybean produced larger dry matter, but the increase was negated by decreased harvest index due to excess foliage and lodging. Mix cropping in this study may increase the productivity by incorporating the merit of indeterminate soybean into determinate soybean production.

This year, the authors conducted 2 experiments: one is the major experiment of mix cropping, and another is yield trials to select genotypes for cultivation environment in Kawatabi. The major experiment planted recombinant hetero lines of indeterminate and determinate soybean in mix cropping (replacement arrangement), and each line in solo cropping. The indeterminate line was also planted in solo cropping to prevent from lodging. The yield trials planted total 353 lines derived from crosses between indeterminate US cultivars and determinate Japanese cultivars. This presentation shows experimental details and some of tentative results in this year.

List of scientific papers in 2016 published by field science group in Graduate School of Agricultural Science, Tohoku University

The Forest-Andisols Group

Xia, Q., M. Ando and K. Seiwa (2016) Interaction of seed size with light quality and temperature regimes as germination cues in 10 temperate pioneer tree species. Functional Ecology, 30: 866-874.

Bayandala, Y. Fukasawa and K. Seiwa (2016) Roles of pathogens on replacement of tree seedlings in heterogeneous light environments in a temperate forest: a reciprocal seed sowing experiment. Journal of Ecology, 104: 765-772.

Ikezaki, Y., Y. Suyama, B. A. Middleton, Y. Tsumura, K. Teshima, H. Tachida and J. Kusumi (2016) Inference of population structure and demographic history in Taxodium distichum, a coniferous tree in North America, based on amplicon sequencing analysis. American Journal of Botany, 103: 11-13.

Nakamura, K., T. Yano, Y. Suyama, O. Nishimura and K. Nakano (2016) Macrofauna in a full-scale vertical flow constructed wetland during vegetative growth stage. Japanese Journal of Water Treatment Biology, 52: 45-54.

Sugai, K., S. Watanabe, T. Kuishi, S. Imura, K. Ishigaki, M. Yokota, S. Yanagawa and Y. Suyama (2016) Extremely low genetic diversity of the northern limit populations of Nypa fruticans (Arecaceae) on Iriomote Island, Japan. Conservation Genetics, 17: 221-228.

Fukasawa, Y. (2016) Seedling regeneration on decayed pine logs after the deforestation events by pine wilt disease. Annals of forest research (in press)

Fukasawa, Y., Y. Yoshihara and Y. Matsuki (2016) Aboveground-belowground linkages. Tokai University Press, Kanagawa (translated to Japanese from Bardgett and Wardle (2010) Aboveground-belowground linkages. Oxford University Press, Oxford).

Fukasawa, Y. (2016) Biodiversity in deadwood. Bulletin of the mountain naturalist club, 15: 73-77.

Takahashi, T. and R. A. Dahlgren (2016) Nature, properties and function of aluminum-humus complexes in volcanic soils. Geoderma, 263: 110-121.

Matsui, T., T. Takahashi, H. Kanno and M. Nanzyo (2016) Inhibitory effect of amorphous aluminum hydroxide gels on organic carbon decomposition in non-allophanic Andosols. Pedologist, 60(1): 4-13.

The Ruminant Production Group

Chen, S., S. Ogura and S. Sato (2016) Effect of environmental enrichment on pituitary gland hormones and behavior of cattle. Abstract of the 14th International Symposium of Integrated Field Science, pp. 10–11 (invited).

Futahashi, R., M. Yayota, N. Hatakeyama, T. Shishido and S. Ogura (2016) Application of a wearable camera to analyze ingestive behavior of grazing cattle. Abstract of the 14th International Symposium of Integrated Field Science, pp. 21.

Hatakeyama, N., R. Futahashi, T. Shishido and S. Ogura (2016) Foraging behavior of cattle in a diverse, mountainous grazing land: Bite size estimation of plants by hand-plucking method. Abstract of the 14th International Symposium of Integrated Field Science, pp. 22.

Jang, SY., S. Ogura, K. Kawamura, M. Yayota, YS. Yun, HJ. Seong, SH. Moon and BT. Jeon (2016) Seasonal changes in forage and animal productivity of Korean native goats grazed at different forage type. Abstract of the 14th International Symposium of Integrated Field Science, pp. 6 (invited).

Kawamura, K., JH. Lim, M. Yayota, S. Ogura, SY. Jang and SH. Moon (2016) Spatial heterogeneity in grazing pasture from small unmanned aerial vehicle (sUAV) with structure from motion (SfM) photogrammetry. Abstract of the 14th International Symposium of Integrated Field Science, pp. 12 (invited).

Lim, JH., K. Kawamura, R. Yoshitoshi, M. Yayota and S. Ogura (2016) Hyperspectral assessment for legume contents and forage nutrient status in pasture. Abstract of the 14th International Symposium of Integrated Field Science, pp. 13(invited).

Ogura, S., S. Sato, S. Tanaka, T. Shimizu and A. Kodera (2016) Effect of cattle grazing in a wasted mulberry plantation on productivity of mulberry leaves and nutritional condition of cattle. Japanese Journal of Grassland Science, 61: 213-223 (in

Japanese with English summary).
Ogura, S. and S. Tanaka (2016) Pasture establishment in a wasted mulberry plantation under cattle grazing: Effect of seeding rate and bare area size on the establishment of sown species. Japanese Journal of Grassland Science, 62(2), 70-74 (in Japanese with English summary).
Ogura, S., H. Mizuno, S.Takamizawa, M. Yayota and K. Kawamura (2016) Nutritional characteristics of forbs and tree leaves and their contribution to animal production in species-rich vegetation. Abstract of the 14th International Symposium of Integrated Field Science, pp. 5 (invited).
Takamizawa, S., T. Shishido and S. Ogura (2016) Diet composition and nutrient uptake of cattle in a pasture-forest combining grazing area of northeast Japan. Proceedings of 10th International Rangeland Congress, pp. 1171-1173.
Takamizawa, S., T. Shishido and S. Ogura (2016) Effect of native grasses, forbs and trees on nutrient uptake of grazing cattle in a temperate region of Japan. Proceedings of 10th International Rangeland Congress, pp. 1174-1176.
Tamiya, S., K. Doi, N. Nakajima, S. Ogura and M. Yayota (2016) Effect of plant diversity on ruminal degradability of goats grazing in a semi-natural pasture. Abstract of the 14th International Symposium of Integrated Field Science, pp. 19.
Takamizawa, S., T. Shishido and S. Ogura (2016) Does species richness of diet affect ruminal digestion characteristics of plants? -A preliminary study. Abstract of the 14th International Symposium of Integrated Field Science, pp. 20.
Tozawa, A. (2016) Contribution of rearing at pasture on improvement of animal welfare in fattening pigs. Abstract of the 14th International Symposium of Integrated Field Science, pp. 9 (invited).
Tozawa, A., T. Takahashi and S. Sato (2016) Effects of pasturing system on behaviour, lung disease, enteric bacteria and back fat skatole in fattening pigs. Tohoku Journal of Animal Science and Technology, 66(1), 23-32 (in Japanese with English summary).
Tozawa, A. and K. Takeda (2016) The use of artificial grass to attract wild sika deer. Proceedings of the 50th Congress of the International Society for Applied Ethology, pp. 237.
Yonezawa, C., S. Ogura and M. Saito (2016) Monitoring for the spatial distribution of radiation dose by paramotor observation. Journal or the Japanese Agricultural Systems Society, 32(3), 95-102 (in Japanese with English summary).
Yonezawa, C., S. Ogura and M. Saito (2016) Aerial measurement of radiation dose distribution on grassland area in Kawatabi Field Science Center. Journal of Integrated Field Science, 13, pp.45 (Abstract of the 13th International Symposium of Integrated Field Science).
Yonezawa, C., S. Ogura and M. Saito (2016) Monitoring temporal vegetation changes on ungrazed grassland by satellite and paramotor remote sensing. Abstract of the 14th International Symposium of Integrated Field Science, pp. 24.
Ogura, S. (2016) A long-term ecological research in a *Miscanthus* grassland, Kawatabi area. Japanese Journal of Grassland Science, 62(2), 87-90 (in Japanese).
Tozawa, A. (2016) The advantages of fattening pigs rearing at pasture. Japanese Journal of Grassland Science, 62(1), 33-37 (in Japanese).
Tozawa, A. (2016) Behavioural and physiological responses of fattening pigs rearing at pasture. Proceedings of Japanese Society for Animal Nutrition and Metabolism, 60(1), 45-51 (in Japanese).
Tozawa, A. (2016) Behavioural expressions and the effects of fattening pigs rearing at pasture. Yoton-no Tomo Nov.2016, 13-15 (in Japanese).
Nguyen, S.T., Y. Fukuda, D.T. Nguyen, C. Tada and Y. Nakai (2016) Prevalence and first genotyping of *Giardia duodenalis* in beef calves in Vietnam. Tropical Animal Health and Production, 48: 837–841.
Yoshihara, Y., C. Tada, M. Takada, N. O. Purevdorj, K. Chimedtseren and Y. Nakai (2016) Effects of water source on health and performance of Mongolian free-grazing lambs. Small Ruminant Research, 137: 81–84.
Baba, Y., Y. Matsuki, Y. Mori, Y. Suyama, C. Tada, Y. Fukuda, M. Saito, and Y. Nakai (2016) Pretreatment of lignocellulosic biomass by cattle rumen fluid for methane production. Bacterial flora and enzyme activity analysis. Journal of Bioscience and Bioengineering (in press).
Nakamura, K., R. Hatakeyama, N. Tanaka, K. Takisawa, C. Tada, and K. Nakano (2016) A novel design for a compact constructed wetland introducing multi-filtration layers coupled with subsurface superficial space. Ecological Engineering (in press).

Nakai, Y. and Y. Fukuda (2016) Cryptosporidiosis. Zoonosis, Third Ed, Eds: Kimura, T. et al., pp.420–424 (in Japanese).

Nakai, Y. (2016) Introduction of Advances of Tohoku University Rapeseed Project, Eds: Nakai, Y. et al., Tohoku University Press (in press), (in Japanese).

Nakai, Y. (2016) Renewable energy and rapeseed, Advances of Tohoku University Rapeseed Project, Eds: Nakai, Y. et al., Tohoku University Press (in press), (in Japanese).

Nakai, Y. (2016) Future of Tohoku University Rapeseed Project, Advances of Tohoku University Rapeseed Project, Eds: Nakai, Y. et al., Tohoku University Press (in press), (in Japanese).

Nakai, Y. (2016) concluding remarks of Advances of Tohoku University Rapeseed Project, Eds: Nakai, Y. et al., Tohoku University Press (in press), (in Japanese).

Nakai, Y., T.Nishio, H. Kitashiba, M. Nanzyo, M. Saito, T. Ito, M. Ohmura and Y. Kanayama (2016) Tohoku University rapeseed project for restoring tsunami-salt-damaged farmland -Was the wisdom of agricultural science utilized for the restoration?- Springer (in press).

Nakai, Y. (2016) Environmental preservation and laws and regulations, Animal Rearing Management, Eds: Kamata, T. et al., Buneido Press (in press).

Nakai, Y. (2016) Amount and characteristics of animal manure, Animal Rearing Management, Eds: Kamata, T. et al., Buneido Press (in press).

Nakai, Y. (2016) Animal manure treatment by microbial systems, Dictionary of food and microbes, Eds: Saito, K. et al., Asakura Publishing (in press).

Tada, C., T. Kotogaoka, M. Takada and Y. Nakai (2016) Monitoring of microbial pathogens in pasturing livestock around Tuul river in Mongolia, Journal of Miyagi Prefectural Veterinary Medical Association (in press), (in Japanese).

Nakai, Y. (2016) Animal environment HACCP and plant factories, Sustainable livestock production and human welfare, 70(12), 919-932 (in Japanese).

Nakai, Y. (2016) Issues in the animal environment and future, Livestock Industry’s Environmental Information, 65, 1-6 (in Japanese).

Nakai, Y. et al. (2016) Growth of production by compost: Composting suitable for farming and distribution, Livestock Industry’s Environmental Information, 66, 13-18 (in Japanese).

Nakai, Y. (2016) Reconstruction of farmlands, food, agriculture and villages, Governance, Gyosei, Maruch issue, No.179, 27-29 (in Japanese).

Nakai, Y. (2015) Sound human in sound environment, Sustainable livestock production and human welfare, 69(12), 1010-1020 (in Japanese).

Nakai, Y., T. Nishio, H. Kitashiba, M. Nanzyo, M. saito, T. Ito, M. Ohmura, M. Abe and Y. Ogushi (2015) The Agri-Reconstruction Project and Rapeseed Project for Restoring Tsunami-Salt-Damaged Farmland after the GEJE – An institutional effort, Post-Tsunami Hazard: Restoration and Reconstruction. In: Advances in Natural and Technological Hazards Research, 44, pp.293-310, Springer.

Nakai, Y. (2015) Sound human in sound environment, Sustainable livestock production and human welfare, 69(12), 1010-1020 (in Japanese).

The Rice Production Group

Asagi, N., K. Minamide, T. Uno, M. Saito and T. Ito (2016) Acidulocompost, a food waste compost with thermophilic lactic acid fermentation: its effects on potato production and weed growth. Plant Production Science, 19: 132-144.

Gao, X., T. Ito, H. Nasukawa and S. Kitamura (2016) Application of Fertilizer Made of Steelmaking Slag in the Recovery of Paddy Fields Damaged by the Tsunami of 2011. ISIJ International, 56: 1103-1110.

Hara, S. and M. Saito (2016) Isolation of inositol hexaphosphate (IHP) degrading bacteria from arbuscular mycorrhizal fungal hyphal compartments using a modified baiting method involving alginate beads containing IHP. Microbes and Environment, 31: 234-243.

Nira, R. and T. Ito (2016) Formulation of a basic guideline to reduce phosphate fertilizer for paddy rice. Japanese Journal of Soil Science and Plant Nutrition, 87: 462-466 (in Japanese).

Kato, Y., R. Tajima, A. Toriumi, K. Homma, N. Moritsuka, T. Shiraiwa, J. Yamagishi, P. Mekwatanakern, V. Chamarerk and B. Jongdee. (2016) Grain yield and phosphorus uptake of rainfed lowland rice under unsubmerged soil stress. Field Crops Research, 190: 54–59.

Yamaguchi, N., I. Taniyama, T. Kimura, K. Yoshioka and M. Saito (2016) Contamination of agricultural products and soils with radiocesium derived from the accident at Fukushima Daiichi Nuclear Power Station: Monitoring, case studies and countermeasures. Soil Science and Plant Nutrition, 62: 303-314.

Yonezawa, C., S. Ogura and M. Saito (2016) Monitoring for the special distribution of radiation dose by paramotor observation. Journal of the Japanese Agricultural Systems Society, 32: 95-102 (in Japanese).

Asakawa, S., K. Suekuni, D. Liu, K. Akita, T. Uno, R. Tajima, T. Ito and M. Saito (2016) Effect of winter-flooding on methanogenic archaeal community structure in paddy field under organic farming. Agriculture and Horticulture, 91: 129-139 (in Japanese).

Ito, T. and K. Akita (2016) Effect of winter-flooding on soil nutrient dynamics and methane emission in organically managed paddy fields. Agriculture and Horticulture, 91: 167-172 (in Japanese).

Saito, M. (2016) Future perspectives on LCA in agriculture: Lessons from the research in Japan, Seminar at PhilRICE (17-18/Feb/2016).

Saito, M., R. Tajima and S. Uchida (2016) Possibility of non-nodulating soybean cultivation from the viewpoint of phosphorus resource scarcity, Ecobalance 2016 International Conference, Kyoto.

Tajima, R., K. Takahashi, T. Umetsu, T. Ito and M. Saito. (2016) Predicting yield, flowering and harvesting dates of highbush blueberry using temperature data: a case study in Field Science Center of Tohoku University. The 13th International Symposium on Integrated Field Science. Sendai.

Yonezawa, C., S. Ogura and M. Saito (2016) Spatial distribution of radiation dose in grassland area on Kawatabi Field Science Center, 13th International Symposium on Integrated Field Science, Sendai.

Yonezawa, C., S. Ogura and M. Saito (2016) Monitoring Temporal Vegetation Changes on Ungrazed Grassland by Satellite and Paramotor Remote Sensing, 13th International Symposium on Integrated Field Science, Sendai.

Marine Bio-Production Group

Hirase, S., M. Ikeda, S. Hayasaka, W. Iwasaki and A. Kijima (2016) Stability of genetic diversity in an intertidal goby population after exposure to tsunami disturbance. *Marine Ecology,* 37: 1161-1167.

Ando, D., M. Ikeda, M. Sekino, T. Sugaya, D. Katamachi, K. Yoseda and A. Kijima. (2016) Improvement of mitochondrial DNA haplotyping in Japanese flounder populations using the sequences of control region and ND2 gene. *Nippon Suisan Gakkaishi,* 82: 712-719. (in Japanese with English abstract)

Hasegawa, M., M. Ikeda and Y. Fujimoto (2016) Distribution of the exotic freshwater shrimp *Neocaridina spp*. in Miyagi Prefecture and influence on the habitat of native shrimp *Paratya compressa improvisa. Izunuma-Uchinuma Wetland Researches,* 9: 47-56. (in Japanese with English abstract)

Kurita, Y., N. Hashimoto and W. Hiroshi (2016) Evolution of the molluscan body plan: the case of the anterior adductor muscle in bivalves. *Biological Journal of the Linnean Society,* 119: 420-429.

Okawa, T., Y. Kurita, K. Kanno, A. Koyama and N. Onikura (2016) Molecular analysis of the distributions of the invasive Asian clam, *Corbicula fluminea* (O.F. Müller, 1774), and threatened native clam, C. leana Prime, 1867, on Kyushu Island, Japan. *Bioinvasions Records*, 5: 25-29.

Kurita, Y. and N. Onikura (2016) Phenotypic shift of an alien piscivorous chub following translocation from a large lake to small irrigation ditches. *Ecological Research,* 31: 731-738.

Integrated Field Control Group

Morita, K. (2016) Consideration of Business Organizations of Family Farm, A Result Paper for Grants-in-Aid for Scientific Research of JSPS, pp.13-22 (in Japanese).

Morita, K. and M. Takahashi (2016) World Food Problems, Food Economics 5th ed., pp.158-179 (in Japanese).

Morita, K. and Y. Ohya (2016) Food Policy in Japan, Food Economics 5th ed., pp.180-196 (in Japanese).

Omura, M., H. Kihara, H. Hara and H. Bannai (2016) Current Situation and Challenging Issues in Restoration after 5 Years since the 2011 GEJE. Report on the Reconstruction Support in Higashi Matsushima, Rural Finance Research Institute: 1

Yonezawa, C., S. Ogura and M. Saito (2016) Aerial Measurement of Radiation Dose Distribution on Grassland Area in Kawatabi Field Science Center, BJournal of Integrated Field Science, 13, 45 (abstract).

Yonezawa, C., S. Ogura and M. Saito (2016) Extraction of vegetation change on the grass land using paramotor-acquired imagery, Proceedings of the 60th Spring conference of the remote sensing society of Japan: 137-138 (in Japanese with English abstract).

Yonezawa, C. and M. Watanabe (2016) MONITORING OF VARIABILITY IN CROP GROWTH ON RECONSTRUCTED AGRICULUTURAL LAND AFTER THE 2011 GREAT EAST JAPAN EARTHQUAKE, Proceedings of International Geoscience and Remote Sensing Symposium 2016, 6308-6311, DOI: 10.1109/IGARSS.2016.7730649.

Yonezawa, C. (2016) Observation of agricultural area on semi-mountainous area using Pi-SAR-L2, Proceedings of the 2016 Fall conference of the Japanese Agricultural Systems Society, 35-36 (in Japanese).

Sekizawa, A., C. Yonezawa, D. Takahashi, K. Nagasawa and M. Osada (2016) Estimation of chlorophyll alpha contents using remote sending data, Proceedings of the 2016 Fall conference of the Japanese Agricultural Systems Society, 27-28 (in Japanese).

Yonezawa, C. (2016) Comparison of an ALOS-2 / PALSAR-2 decomposition image with a SPOT image for crop type classification, Proceedings of the 61th Fall conference of the remote sensing society of Japan: 143-144 (in Japanese with English abstract).

Yonezawa, C., S. Ogura and M. Saito (2016) Monitoring temporal vegetation changes on ungrazed grassland by satellite and paramotor remote sensing, The 14th International Symposium on Integrated Field Science –International Symposium on Integrated Field Science - (oral presentation).

Yonezawa, C., S. Ogura and M. Saito (2016) Aerial measurement of radiation dose distribution on grassland area in Kawatabi Field Science Center, The 13th International Symposium on Integrated Field Science – Conservation and Utilization of Biodiversity- (oral presentation).

Yonezawa, C. (2016) An experimental study for agricultural field classification using air-borne synthetic aperture radar in Kawasaki-cho, Miygagi-ken, Research meeting of GIS association of Japan, Tohoku branch (oral presentation).

Yonezawa, C., S. Ogura and M. Saito (2016) Monitoring for the spatial distribution of radiation dose by paramotor observation, Journal of the Japanese Agricultural Systems Society, 32,3: 95-102 (in Japanese with English abstract).

Watanabe, M., C. Yonezawa, J. Sonoda, and M. Shimada (2016) Detection of damaged urban areas using interferometric SAR coherence change with PALSAR-2, Earth, planets and space, 68, 131: 33-34 DOI 10.1186/s40623-016-0513-2.

Sonoda, J., M. Watanabe, C. Yonezawa, Y. Kanazawa, T. Goukon, S. Igarashi and A. Mitsutome (2016) Development of missing persons searching system using ground-penetrating and air-borne radars for large-scale natural disaster and its application to the victims by the Tohoku earthquake on 2011. Proceedings of 18th conference for Japan society for disaster information studies (poster presentation), (original title in Japanese).

Sonoda, J., M. Watanabe, C. Yonezawa and Y. Kanazawa (2016) Experiment of Underground object Detection Using L-band Airbone Radar and UAV-SfM, Proceedings of the 2016 IEICE Society Conference.

Watanabe, M., C. Yonezawa, J. Sonoda and M. Shimada (2016) Trial of detection for damaged urban area and landslide area from wide range of image obtained by L-ban SAR (PALSAR-2), the 60th Spring conference of the remote sensing society of Japan (poster presentation).

Yonezawa, C. (2016) Development of Database for Kawatabi Field Center, Bulletin of Integrated Field Science Center, 31: 1-7 (in Japanese).

Yonezawa, C. (2016) GIS data conversion of crops temporal change map on Field Science Center, Bulletin of Integrated Field Science Center, 31: 77-101 (in Japanese).

Information to Authors from Journal of Integrated Field Science (JIFS)

JIFS publishes articles in all areas of field science in Agricultural science. This journal is an English magazine started in 2003 fiscal year when Integrative Field Science Center, Graduate School of Agricultural Science, Tohoku University, started.

Our journal places the edit committee. Under the committee, original papers including short paper, proceedings, reviews, descriptions, and data are published. They undergo two reviser's (referee) examination.

In principle JIFS is published annually. We publish it as a printing matter and all of the manuscripts are available online.

Manuscript creation point

Please write your contribution paper according to the paper format of our journal; (1) title, (2) running title, (3) affiliation and its address, (4) e-mail address of corresponding author, (5) key words (five to seven words not appearing in the title), (6) abstract (about 250 words), (7) introduction, materials and methods, results, discussions (results and discussions), (acknowledgement), references, table files, figure files, legend of figures are included.

Your paper should be written in A4 size paper in Word file or text file, tables should be drawn by excel file and figures in tiff or jpeg file or Power Point file, respectively. Please send your paper in word format to our editorial board via e-mail.

Submission Deadline: November 30, 2017.

Next Publication : JIFS vol.15 will be published in March, 2018.

Editorial Board
Chief Editor: Shin-ichiro Ogura (Japan)
Members: Randy A. Dahlgren (USA), Joji Iisaka (Canada), Qi Li (China), Attila Ombodi (Hungary), Lilik Budi Prasetyo (Indonesia), Steven Mark Rutter (UK), Myung Gyu Lee (Korea), Akihiro Kijima (Japan), Makoto Osada (Japan), Yutaka Nakai (Japan), Masanori Saito (Japan), Kenji Seiwa (Japan), Kiyohide Morita (Japan), Chinatsu Yonezawa (Japan).
Secretary: Mutsuko Kobayashi, mutsuko.kobayashi.d6@tohoku.ac.jp
Chiho Nishiwaki, chiho.nishiwaki.a3@tohoku.ac.jp

Address of Editorial Board
Address : Laboratory of Land Ecology, Graduate School of Agricultural Science, Tohoku University
232-3, Yomogida, Naruko-Onsen, Osaki, Miyagi, 989-6711, Japan

Manuscript Sending Address
E-mail : shin-ichiro.ogura.e1@tohoku.ac.jp