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Symposium Paper

Introduction of Biomass Accounting as an Evaluation Tool of Biomass Utilization Systems: a Case Study on Domestic Animal Waste Treatment-Oriented Biomass Activities

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Abstract

Traditionally, primary industry's biomass leftovers had been treated with the purpose of waste reduction. However, nowadays, efficient utilization of biomass resources is regarded as an efficient tool of rural economy revival, zero-emission society establishment, and reduction of greenhouse gas emissions. In Japan, the necessity of biomass utilization for sustainable society was declared in Biomass Nippon Strategy, 2002. Based on this document, many rural towns and villages all around the country has adopted a "Biomass Town" concept and started to develop biomass utilization systems with multiple activities, including material, energy, and education biomass-related projects.

However, no comprehensive evaluation of economic and environmental efficiency of already existing biomass utilization systems is conducted. One of the reasons is that there exist no guidelines, which could assist in such estimation process.

In this paper, first, brief introduction on biomass accounting concept, suggested by authors in our previous works as an accounting tool to assist in decision-making related to biomass activities, is made. Next, the results of a case study on domestic animal waste treatment-oriented biomass activities (composting) are presented. The biomass accounting form is applied in the case study to account for four compost facilities built and managed by local government. Finally, discussion on the possibility of economic and environmental efficiency improvement of the biomass activities under the case study is conducted.

1. Introduction

Biomass resources had been used by humans as energy (direct burning, carbonization) or materials (compost, forage) since ancient times. Technology development caused shift to fossil fuel and chemical fertilizer consumption, and the main purpose of primary industry's biomass leftover treatment became waste reduction. However, nowadays, efficient utilization of biomass resources is regarded as an important tool of rural economy revival, zero-emission society establishment, and reduction of greenhouse gas emissions. In Japan, the necessity of biomass utilization for sustainable society was declared in Biomass Nippon Strategy, 2002. Based on this document, many rural towns and villages all around the country has adopted a "Biomass Town" concept and started to develop biomass utilization systems with multiple activities, including material, energy, and education biomass-related projects. These activities include traditional biomass treatment technologies, as well as modern ones, including bioplastic and wood block production (material), biodiesel and bioethanol production (energy), biomass events and tours (education), among others.

However, no comprehensive evaluation of economic and environmental efficiency of already existing biomass utilization systems is conducted. One of the reasons is that there exist no guidelines, which could assist in such estimation process. Taking this into account, a biomass accounting concept was suggested in our previous works as an accounting tool to assist in decision-making related to biomass activities.

Fig. 1 presents a biomass accounting framework. It can be divided into “Stock” and “Flow” parts. “Stock” consists of “Initial Cost” and “Natural Capital” and is aimed to record for human-made and natural stock. “Flow” part includes “Running Cost”, “Benefit”, and “Fossil Resources Substitution”. It records for economic and material inputs and outputs during a certain period of time (generally one year). Both “Stock” and “Flow” contain “Economy” and “Environment” areas. “Economy” records actual data on capital asset cost, running cost, and economic benefits. “Environment” records estimated environmental load and effect. “Flow” part contains “Resources” area as well, which traces the amount of material input and output. Biomass accounting framework is discussed in details in Bespyatko et al, 2009. Further, based on the concept of biomass accounting, a biomass accounting form was suggested by authors (Bespyatko et al, 2010). The form was designed in order to simplify data input and to automatize output and visualization of the results.

In this study, the biomass accounting form is applied to account for four compost facilities built and managed by local government. The next two sections explain the methodology and present the results of a case study.

2. Estimation methodology: assumptions and preliminary conditions

For the case study Shobara biomass town in Hiroshima prefecture was chosen. Biomass accounting form was applied in order to estimate economic and environmental performance of four compost facilities, construction of which was subsidized by government. Preliminary calculations of composting-related activities in this biomass town were conducted and presented in Bespyatko et al, 2009. In this study we improve the reliability of the results by re-considering a part of the calculation methodology. Namely, 1) not only CO_2 but such greenhouse gases as CH_4 and N_2O are considered as well to estimate environmental load and effect; 2) environmental load of the fermentation process is taken into account; 3) calculation of asset depreciation is based on actual durable years of capital instead of legislative ones; 4) diesel price is set at 118 yen/l (last 5 years average) instead of 150 yen/l (representative price of 2008).

Regarding the environmental load associated with compost (fermentation process), 0.00044 t-CH_4/t-manure and 0.0039 t-N_2O/t-N set in “Calculation methods and list of emission indices for calculation, reporting, and disclosure systems” (Ministry of Environment, 2010) are used (Table 1).

As to durable years of capital assets, though certain years are set by the law, actually such assets as buildings or machinery are used for much longer period. Because biomass-related activities do not consume large amount of fossil fuels, the most of environmental load coming from such an activity is the environmental load embodied in its initial cost. Hence, it is important to use actual durable years for environmental load estimation wherever possible. Table 2 lists legislative durable years and actual durable years, which were applied in this study. Actual durable years were set based on the information, obtained during hearings and conversations with related stakeholders.

Prices, used to estimate costs and benefits when actual data were not available, are listed in Table 3. For diesel, August 2005 - July 2010 average price of 118 yen/l is taken. Electric power is set at 15 yen/kWh. Regarding compost price, actual data of profit from sales are used; however, for barter exchange and captive use, the price is assumed to be 5,000 yen/t.

<table>
<tr><th rowspan="3">Stock</th><th>Economy</th><th>Environment</th><th rowspan="3">Flow</th><th>Economy</th><th colspan="6">Resources</th><th rowspan="2">Environment</th></tr>
<tr><th rowspan="2"></th><th rowspan="2"></th><th colspan="3">Use/Production</th><th>Within Region</th><th>Outside Region</th></tr>
<tr><th>10,000 yen</th><th>Type</th><th>Quantity</th><th>Unit</th><th>Use/Supply</th><th>Import/Export</th></tr>
<tr><th>10,000 yen</th><th>Load</th><th></th><th></th><th></th><th></th><th>Quantity</th><th>Unit</th><th>Quantity</th><th>Unit</th><th>Load & Effect</th></tr>
<tr><td>1. Initial Cost</td><td colspan="2"></td><td>1. Running Cost</td><td colspan="8"></td></tr>
<tr><td>Building</td><td rowspan="5">Capital asset cost breakdown</td><td rowspan="7">Capital assets' lifecycle environmental load (energy consumed and GHG emitted) ☆Estimated only for capital assets☆</td><td>Material</td><td rowspan="6">Running cost break down</td><td rowspan="6" colspan="3">Total utilization amount of biomass and other resources</td><td rowspan="6">Utilization amount of local resources</td><td rowspan="6" colspan="2">Utilization amount of imported resources</td><td rowspan="6">Energy consumed and GHG emitted during biomass production activities</td></tr>
<tr><td>Truck</td><td>Auxiliary material</td></tr>
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<tr><td></td><td></td><td></td><td>3. Fossil Resources' Substitution</td><td>Substitution Value</td><td colspan="3">Amount of fossil fuels substituted</td><td colspan="4"></td><td>Effect of substitution</td></tr>
</table>

Fig. 1. Biomass accounting framework.

Table 1. Emission indices[1].

Item	Unit	Energy MJ	CO_2 kg	Unit	CH_4 g	N_2O g
Agricultural machinery	10,000 yen	828	65	10,000 yen	84	**
Nonresidential building (nonwooden)	10,000 yen	612	56	10,000 yen	94	**
Car body	10,000 yen	1,060	84	10,000 yen	253	**
Urea	t	9,083	966	t	1,102	35.4
Electricity	kW	10.490	0.544	MW	2.25	1.62
Diesel	kl	41,514	2,812	kl	355	882
Compost (dairy cow manure)	---	---	---	t	440	13.1

[1]Energy consumption, CO_2 and CH_4 emission indices for agricultural machinery, nonresidential building, car body, and urea are taken from JEMAI, 1999. N_2O emission index for urea was not available, hence, N_2O emission index for ammonia is taken from JEMAI-LCA Pro, 2005 (hearing data). Energy consumption, CO_2, CH_4, and N_2O emission indices for electricity and diesel are based on CGER, 2010. Energy and CO_2 embodied in compost is calculated separately by aggregating method in biomass accounting form. In order to calculate the amount of N_2O embodied in 1t of manure, the index of 3.358 g-N/kg manure (LEIO, 1998) was used.

Table 2. Legislative and actual durable years.

	Legislative durable years	Actual durable years[1]
Non-residential building	20	40
Equipment	7	25
Truck	5	15

[1]Actual durable years are set based on information obtained during hearings

Table 3. Prices used for estimation.

	Price
Diesel	118 yen/l
Electric power	15 yen/kWh
Urine	300 yen/kg
Compost (barter, captive use)	5,000 yen/t

Estimation of chemical fertilizer substitution by compost is based on data taken from LEIO, 1998 (Table 4) and hearing data on the manure amount utilized by each facility (Table 5).

3. Results

Total cost-benefit and environmental performance of the four composting facilities under the case study is summarized in the form of accounting table in Fig. 2 and visualized in Graph 1-2.

Totally, 7,601 t of compost is produced from 16,475 t of cow manure annually. Most of the product is sold (4,188 t/year) or distributed freely (indirect captive use, 1,198 t/year). Total amount of urea substituted is 94 t[1]. Economic value of substitution equals to 28,260 thousand yen assuming that urea unit price is 300 yen/kg.

Regarding economic profitability, total benefit was 33,830 thousand yen/year comparing to 27,150 thousand yen/year of running cost. Here, total benefit

Table 4. Cow manure compost element composition and chemical fertilizer substitution rate.

Cow manure compost	T-N	P_2O_5	K_2O
Compost element composition (%)	1.9	2.3	2.4
Chemical fertilizer substitution (%)	30	70	90

Table 5. Manure amount utilized and chemical fertilizer substituted by each facility.

	Facility A			Facility B			Facility C			Facility D		
Manure amount (t/year)	3,200			5,181			1,094			7,000		
	N	P	K	N	P	K	N	P	K	N	P	K
Active element amount (t)	30.40	36.80	38.40	13.30	16.10	16.80	4.94	5.98	6.24	52.25	63.25	66.00
Chemical fertilizer substituted (t)	9.12	25.76	34.56	3.99	11.27	15.12	1.48	4.19	5.62	15.68	44.28	59.40

Stock	Economy	Environment				Flow	Economy	Resources							Environment			
		Environmental load						Use/production			Within region		Outside region		Load and Effect			
	10,000 yen	E	CO2	CH4	N2O		10,000 yen				Use/Supply		Import/Export		Energy	CO2	CH4	N2O
		MJ	t	kg	kg			Type	Quantity	Unit	Quantity	Unit	Quantity	Unit	MJ	t	kg	kg
1. Initial cost	58,942	40,288,872	3,503	5,310	***	1. Running cost	2,715								4,882,355	311	7,425	235
Building	42,470	25,991,640	2,378	3,992	***	Material	0	Manure	16,475	t		t		t	0	0	7,249	216
Truck	2,838	3,008,280	238	718	***	Auxiliaries	515	Bark	***	m3		m3		m3	0	0	0	0
Equipment	13,634	11,288,952	886	600	***	Fuel	265	Diesel	0	l					928,774	63	7.95	20
Subsidy	33,982					Electricity	379	Electricity	***	kW					2,651,680	137	0.57	0.41
Real initial cost	24,960	40,288,872	3,503	5,310	***	Depreciation	1,613								1,301,901	111	172	***
						(Real)	925											
						Labor	280											
						Other	351											
						2. Benefit	3,383	Compost	7,601	t		t		t				
						Direct	1,905											
						Sales	1,541	Compost	4,188	t	4,188	t	0	t				
						Barter Exchange	0	---	---	---	---	---	---	---				
						Captive use	0	---	---	---	---	---	---	---				
						Acceptance fee	364	Manure	4,195	t	4,195	t	0	t				
						Indirect	1,478											
						Sales	0	---	---	---		---		---				
						Barter Exchange	280	Compost	560	t	560	t	0	t				
						Captive use	1,198	Compost	2,853	t	2,853	t	0	t				
						3. Substitution	2,826	Urea	94	t	94	t	0	t	-855,494	-91	-104	-3.33
						Depasturage emissions	*0*	*Manure*	*16,475*	*t*					*0*	*0*	*-1,300*	*-180*

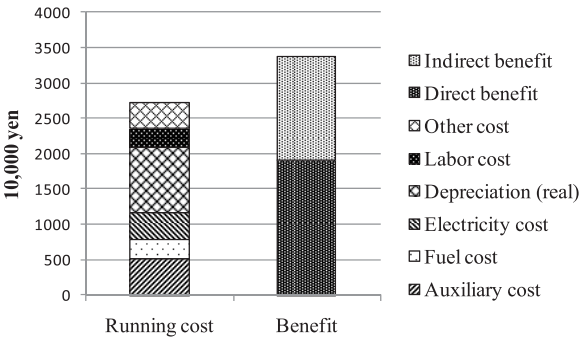
Fig. 2. Total economic and environmental performance of the four composting facilities.

includes direct and indirect benefit[2]. However, if consider only direct benefit (19,050 thousand yen), the activity turns to be unprofitable. Yet, because there is a considerable (hidden) economic gain from indirect profit (such as barter exchange and captive use), which is usually not recorded in orthodox accounting tools, we suggest that it has to be paid attention and included into calculations as well.

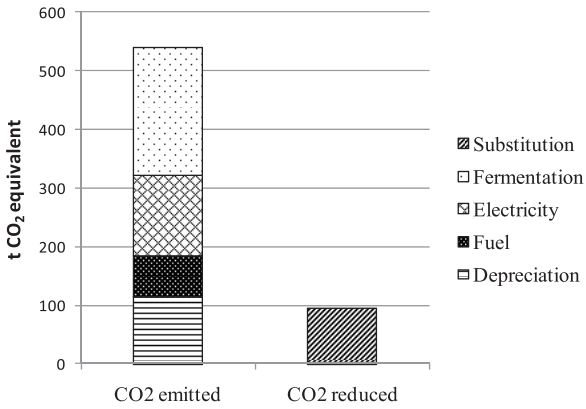
From the table and graphs it is clear that total environmental performance of the biomass activities under the case study is poor. Total emission is 540 t of CO_2 equivalent per year, which is much higher than total CO_2 reduction from urea substitution (94 t CO_2 equivalent/year). Most of the emissions are coming from manure fermentation process (219 t CO_2 equivalent/year), electricity consumption (138 t CO_2 equivalent/year), and depreciation (114 t CO_2 equivalent/year). From this, it becomes clear that en-

[1] Based on Table 4-5, amount of nitrogen containing in 7,601 t of compost is calculated to be 43 t (chemical fertilizer substitution coefficient (30%) is considered). This substitutes 94 t of urea assuming that urea nitrogen content is 46%.

[2] Direct benefit: benefit gained by an economic activity from the same activity. For example, product sale or consumption of a part of the product in order to produce more of the same product.
Indirect benefit: benefit, which is brought by an economic activity to another economic activity. For example, captive use of pellets with a purpose other than to produce more pellets.



Graph 1. Total cost-benefit performance of the four compost facilities



Graph 2. Total GHG balance of the four compost facilities

Table 6. Environmental load embodied in composting, urea production, and depasturage[1].

	CO_2 (t)	CH_4 (t)	N_2O (t)	Total (t) CO_2 equivalent
Compost (1)[2]	311	7.425	0.235	540
Compost (2)	111	7.249	0.216	330
Urea	91	0.104	0.003	100
Depasturage	0	1.300	0.180	83

[1]Utilized or substituted manure amount is the same for each process (16,475 t)
[2]Compost(1) is composting technology of the facilities under the case study
Compost(2) is possible composting technology which consumes renewable energy

vironmental load of compost facilities under the case study is much higher comparing to that one of urea production or depasturage (Table 6).

4. Discussion

The results of the case study briefly described in the previous section showed that total economic performance of the four facilities is considerably high if indirect benefits are taken into account. However, they also indicated low environmental efficiency of the composting technology under investigation.

One of the possibilities of environmental efficiency improvement of the biomass activities under the case study is shift to renewable energy consumption. Assuming that amount and cost of biofuel (bioethanol or biodiesel) and green electricity necessary for compost production is the same as that one of fossil fuel and electricity which is consumed at present, total environmental load could be reduced from 540 t CO_2 equivalent/year to 330 t CO_2 equivalent/year (Table 6).

Another option is to increase the concentration of nitrogen in compost. Table 7 presents urea amount, profit and CO2 reduced due to substitution of chemical fertilizer with compost with different nitrogen concentration. Here, substitution profit is calculated based on assumption that production cost is not influenced by nitrogen-enhancing technology. From the table it is clear that increasing of nitrogen concentration to 5%-10% (wet value) leads to considerable improvement in economic and environmental efficiency of compost production.

Taking the above into consideration, it can be suggested that shift to renewable energy consumption and increase of nitrogen concentration in compost can considerably raise economic benefit, as well as significantly lower environmental load by means of larger amount of chemical fertilizer substitution. However, the cost of nitrogen-enhancing technology has to be taken into account, as well as more precise estimation of environmental load embodied in urea production need to be conducted to increase the reliability of estimation result. This is the topic for our further research.

Table 7. Influence of nitrogen concentration on compost profitability and environmental efficiency

Nitrogen content (wet value %)	Total nitrogen amount (t)	Urea substituted (t)	Substitution profit (10,000 yen)	CO_2 reduction (t CO_2 equivalent)
0.35	27	17	520	17
1.90	43	94	2,826	94
5	380	248	7,436	248
10	760	496	14,872	496

References

Bespyatko, L. Y., K. Saga, T. Yanagida, W. Aung, S. Fujimoto and T. Minowa (2010) Research on a biomass accounting form with an accent on biomass towns in Japan, Proceedings of 5th Biomass Conference, Lyon, France, 178-179.

Bespyatko, L. Y., C. Tada, T. Yanagida, K. Saga, E. Bautista, S. Fujimoto and T. Minowa (2009) Research on a Biomass Accounting Framework as a Biomass Town Assessment and Information Provision Tool. J. Jpn. Inst. Energy, 88: 1081-1094.

CGER (2010) National Greenhouse Gas Inventory Report of Japan.
http://www-gio.nies.go.jp/aboutghg/nir/nir-j.html (date of access: August 10, 2010)

JEMAI (1999) Introduction to LCA Practic, JEMAI, pp. 194.

JEMAI (2005) JEMAI-LCA Pro, CD data.

LEIO (1998) Livestock manure treatment and utilization guide, LEIO, pp. 202.

Ministry of Agriculture, Forestry and Fisheries (2006) Biomass-Nippon Strategy.
http://www.maff.go.jp/biomass/eng/biomass_honbun.htm (date of access: August 13, 2010)

Ministry of Environment (2010) Calculation methods and list of emission indices for calculation, reporting, and disclosure systems.
http://www.env.go.jp/earth/ghg-santeikohyo/material/itiran.pdf (date of access: August 26, 2010)

Forage Production, Utilization and Environmental Conservation in Sweden

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Abstract

There are totally 1.5 million ha of grassland in Sweden of which 70 percent is temporary in rotations on arable land. Temporary grassland is the dominating crop with 40 percent of the arable cropping area. The climate is cold temperate and winter damages are common in the grasslands. In the North, where farming is done up to 66˚N latitude, light conditions are extreme, which gives higher energy content in the forage. Timothy (*Phleum pratense* L.), meadow fescue (*Festuca pratensis* L.) and red clover (*Trifolium pratense* L.) are the dominating species. Contrary to the intensive grasslands in Europe, it is very common to use a mixture with grasses and legumes. Silage maize can only be grown in very south where the temperature sum is high enough. The area of silage maize is rapidly increasing due to new varieties. Larger milk yield per cow has increased the requirement for herbage with higher nutrient quality. This has lead to earlier cuts and increasing number of cuts. Due to the long winter season a large part of the forage is conserved as silage. In 2009, Sweden had about 350 000 dairy cows on nearly 6 000 farms. About ten percent of the milk farms have organic production. Efforts to reduce N-losses to the environment are done by developing grazing systems and total feeding rations that increase N utilization. Also slurry injection into grassland has advantages over slurry spreading as it decreases ammonia losses and odour and improves forage hygiene.

Climate

Sweden is situated between the latitudes 55˚N and 69˚N and the climate is cold temperate and winter damages are common in the grasslands, especially in red clover. In the North, light conditions are extreme, which gives higher energy content in the forage. Due to the long winter season a large part of the forage is conserved as silage.

The forage production in Sweden

There are totally 1.5 million ha of grassland in Sweden of which 70 percent is temporary in rotations on arable land (Table 1). This proportion is much higher than in many other European countries (Søegaard *et al.*, 2007). Temporary grassland is the dominating

Table 1. Arable grassland permanent grassland and other arable crops in Sweden 2005-2010 in million ha. Source Swedish Agricultural Board (2010).

Crop	2005	2006	2007	2008	2009	2010*
Cereals	1.01	0.96	0.97	1.09	1.05	0.97
Arable grassland	1.03	1.06	1.08	1.11	1.18	1.21
Permanent grassland	0.51	0.51	0.49	0.46	0.44	0.45
Others	0.67	0.64	0.59	0.43	0.42	0.44
Total area	*3.22*	*3.17*	*3.14*	*3.09*	*3.08*	*3.08*
Permanent grassland, percent	16	16	16	15	14	15

*Preliminary values

crop with about 40 percent of the arable cropping area (Table 2). Interestingly this percentage has steadily increased the last six years. The temporary grasslands in Sweden are mainly established in the spring and cut two-three times during two-four years (Søegaard, 2007). Larger milk yield per cow has increased the requirement for herbage with higher nutrient quality. This has lead to earlier cuts and increasing number of cuts.

Silage maize has been a very minor crop in Sweden so far in area compared to the temporary grassland. The silage maize can only be grown in very south where the temperature sum is high enough. But the last ten years the area under silage maize has increased more than five times as seen in Table 2. An important factor in this change has been new released varieties, which are better adapted to the climate.

Table 2. Arable grassland and other arable crops in Sweden 2001-2010 in million ha. Source Swedish Agricultural Board (2010).

Crop	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010*
Cereals	1.15	1.11	1.13	1.11	1.01	0.96	0.97	1.09	1.05	0.97
Arable grassland	0.93	0.94	0.93	0.93	1.03	1.06	1.08	1.11	1.18	1.21
Others	0.62	0.63	0.61	0.62	0.67	0.64	0.59	0.43	0.42	0.44
Total arable land	*2.69*	*2.68*	*2.67*	*2.66*	*2.70*	*2.66*	*2.65*	*2.63*	*2.64*	*2.63*
Arable grassland, percent	35	35	35	35	38	40	41	42	44	46
Silage maize, thousand ha	3.0	3.9	4.0	5.3	5.8	7.5	10.8	13.1	16.1	16.5

*Preliminary values

The number of species used in the Swedish grasslands are high, especially the number of grass species (Table 3). Species marked with M are main species in Sweden according to Søegaard (2007). These species are also dominating in Norway and Finland. When moving more south in Europe, perennial ryegrass and white clover then becomes the main species. Among the grasses, there is an increasing use of perennial ryegrass and festulolium in Sweden, especially in more favourable locations.

The type of farming varies a lot in Sweden, which is illustrated by Figure 1. Most grassland on arable land (28 %) is found is on the high lands in the south Sweden, indicated by animal production dominating symbol on the map. This area, which goes across Sweden, is popular called the Animal belt.

Table 3. Forage species used in Swedish grasslands

	Perennial forage legume species		**Perennial forage grass species**
M	Red clover (*Trifolium pratense* L.)	M	Timothy (*Phleum pratense* L.)
M	White clover (*Trifolium repens* L.)	M	Meadow fescue (*Festuca pratensis* Hudson.)
	Lucerne (*Medicago sativa* L.)		Perennial ryegrass (*Lolium perenne* L.)
	Alsike clover (*Trifolium hybridum* L.)		Festulolium (*Festulolium*)
	Bird's foot trefoil (*Lotus corniculatus* L.)		Hybrid ryegrass (*Lolium x boucheanum*)
			Italian ryegrass (*Lolium multiflorum* Lam.)
			Cocksfoot (*Dactylis glomerata* L.)
			Kentucky bluegrass (*Poa pratensis* L.)
			Red fescue (*Festuca rubra* L.)

M= main species

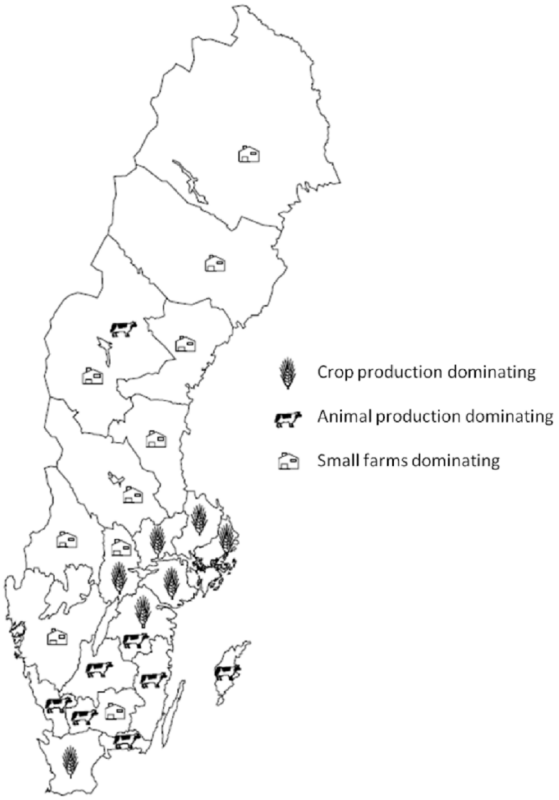

Fig. 1. Dominating type of farms in different regions in Sweden. From Statistics Sweden (2010) page 44.

The dairy and animal production in Sweden

In 2009, Sweden had 357 000 dairy cows on 5 883 farms (Table 4), which means that an average farm had about 60 dairy cows in 2009. There has been for a long time a change to larger farms. The amount of organic produced milk is increasing and in January 2010 about ten percent of the milk farms have organic production (Table 4).

In Table 5, the number of bovine animals, sheep's and lambs in 2007 are reported. Note the decrease in number of dairy cows between 2009 and 2007 (Table 4 and 5).

Seed mixtures in grasslands

Contrary to the intensive grasslands in Europe, it is very common to use a mixture with grasses and legumes in Sweden. In Table 6, two examples are given of mixtures from a seed company in Sweden (SW Seed, 2008). SW 932 in this example is robust mixture for cutting leys with 2-3 harvests per year. SW Ares is a new sustainable variety bread for southern Sweden. SW 843 on the contrary, is a mixture for an intensive and high production meaning 3-4 harvests per year ore combined with grazing. It has two varieties of perennial ryegrass, two of timothy and two of meadow fescue, which are of different types and make the mixture more flexible. Diversity of clover species in grass-clover swards can contribute in first hand to the stability of yield, but does not always mean greater herbage yields (Frankow-Lindberg *et al.*, 2009).

Natural grasslands

The area of semi-natural grasslands in Sweden has been decreasing for a long time in Sweden. In a survey, based on old maps, it was calculated that dur-

Table 4. Structure of dairy sector in Sweden in January 2010. Source: The Swedish Dairy Association (2010).

Description	Value
Number of dairy companies	21
Number of processing plants	36
Number of livestock cooperatives	7
Number of semen-producing companies	2
Number of breed societies	9
Number of dairy farmers	5 883
of which organic	512
Number of dairy cows (June 2009, thousands)	357
Average of cows per herd	59
Total milk delivered Mkg (2009)	2926

Table 5. Livestock numbers in 1 000 head in year 2007

Description	1 000 head
Dairy cows	370
Suckler cows	186
Total cows	*556*
Heifers, bulls and steers older than one year	516
Calves younger than one year	489
Total bovine animals	*1 560*
Ewes and rams	242
Lambs	267
Total sheep and lambs	*509*

Table 6. Example of two seed mixtures from a seed company in Sweden. The percent is the seed weight in the mixture. The variety name is before the name of the species

SW 843 Intensive South	**SW 932 All round Basic**
3 % Sara red clover	5 % SW Ares red clover
3 % SW Ares red clover	5 % Sara red clover
4 % Ramona white clover	65 % Ragnar timothy
20 % Ragnar timothy	25 % Sigmund meadow fescue
15 % Grindstad timothy	
15 % Sigmund meadow fescue	
10 % Tyko meadow fescue	
10 % Malta perennial ryegrass	
10 % Leia perennial ryegrass	
10 % Storm hybrid ryegrass	

ing the last 200 years in 12 landscapes in South-east Sweden, the land-cover of semi-natural grasslands has decreased from 83% to 17% (Cousins, 2009). In a field survey, the semi-natural grasslands in these 12 landscapes had a species richness of 9-29 species m^2.

Environmental conservation

The ecological value and the food production possibilities of the agricultural landscape and the agricultural land are protected in Sweden. In 1999, the Swedish Parliament adopted 15 national environmental quality objectives, and one more has been added since then. These objectives describe what characteristics the natural and cultural environment must have for a more sustainable development of the society. Among the 15 goals, these three objectives apply to the agriculture:

- a varied agricultural landscape
- zero eutrophication
- a non-toxic environment.

These three objectives include the following:

– Existing biodiversity must be preserved by long-term management strategies.

– Small biotopes are to be preserved and also created in the plain districts.

– Nutrient leaching and use of chemical plant protection products shall be reduced.

The objectives shall be reached through legislation, financial instruments, information, extension services, and training.

Efforts to reduce N-losses to the environment are done by develop grazing systems and total feeding rations that increase N utilization. To improve the technique of spreading the manure is also important. Slurry injection into grassland has advantages over slurry spreading as it decreases ammonia losses and odour and improves forage hygiene, but it can increase the nitrous oxide (N_2O) production. However, in spite of the reduction in ammonia emissions, Rodhe et al. (2006) reported little or no effect on yield from the injection technique compared with surface spreading. The most common explanation given for this is that damage to the grass sward caused by injector tools balances out the larger amount of ammonium nitrogen left after slurry injection. If the injection equipment as shown in Fig. 2 is used without slurry, a yield reduction up to 3-9% can occur (Rodhe and Halling, 2010).

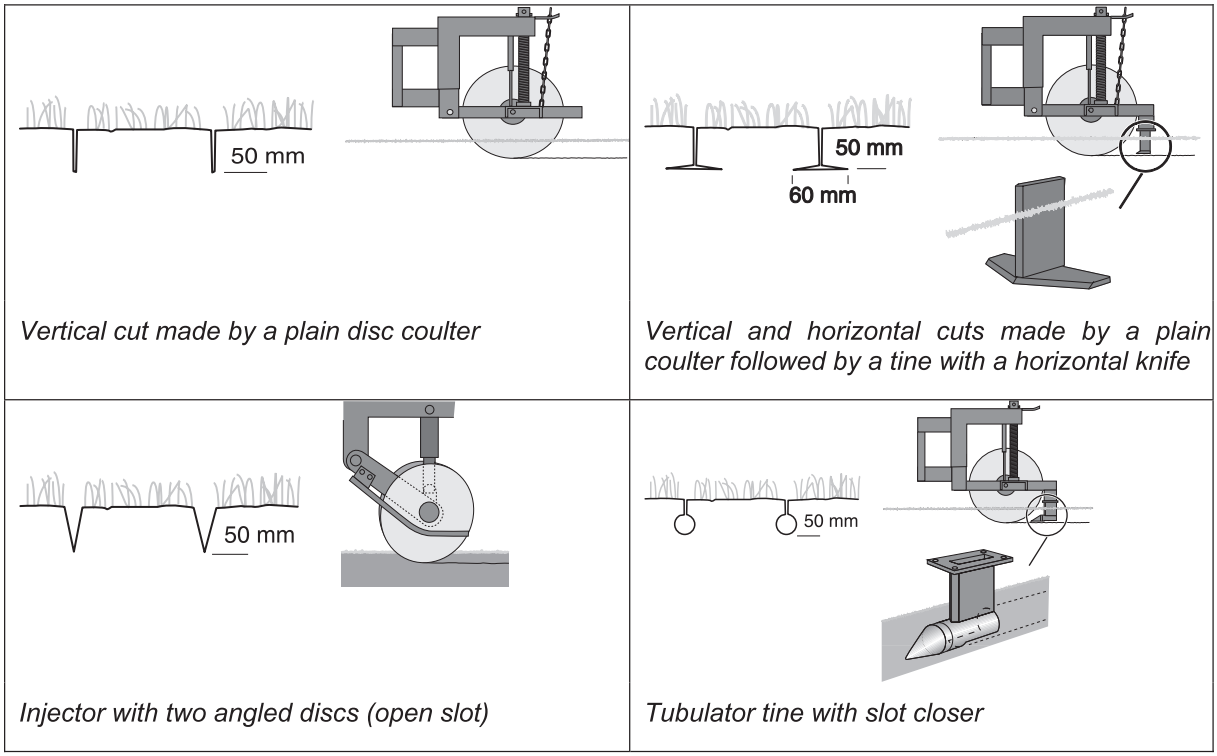

Fig. 2. Different techniques for injecting manure into a grassland sward.

References

Cousins, S.A.O. (2009) Landscape history and soil properties affect grassland decline and plant species richness in rural landscapes. Biological Conservation, 142: 2752-2758.

Frankow-Lindberg B.E., M. Halling, M. Höglind and J. Forkman (2009) Yield and yield stability of single- and multi-legume grass-clover swards in two contrasting environments. Grass and Forage Science, 64, 236-245.

Halling, M. A. and L. Rodhe (2010). Grassland yield response to knife/tine slurry injection equipment – benefit or crop damage? Grassland Science in Europe: 15: 175-177. SW Seed (2008). SW Vallaktuellt – Grassland News 2008.

Rodhe L., M. Pell and S. Yamulki (2006) Nitrous oxide, methane and ammonia emissions following slurry spreading on grassland. Soil Use and Management 22: 229-237.

Søegaard, K., M. Geirus, A. Hopkins and M. Halling (2007) Temporary grassland – challenges in the future. Grassland Sciences in Europe, 12: 27-38.

Statistics Sweden (2010) Yearbook of agricultural statistics 2010 including food statistics. http://www.scb.se/Pages/PublishingCalendarViewInfo____259923.aspx?PublObjId=13495 (date of access: September 11, 2010)

Swedish Agricultural Board (2009) Facts about Swedish agriculture. http://www2.jordbruksver-

ket.se/webdav/files/SJV/trycksaker/Pdf_ovrigt/ovr2gb.pdf (date of access: September 11, 2010)

Swedish Agricultural Board (2010) Action programme for reducing plant nutrient losses from agriculture. http://www2.jordbruksverket.se/webdav/files/SJV/trycksaker/Pdf_ovrigt/ovr138ENG.pdf (date of access: September 11, 2010)

Swedish Agricultural Board (2010) Use of agricultural land in 2010, Preliminary results (JO 10 SM 1002). http://www.sjv.se/webdav/files/SJV/Amnesomraden/Statistik%2C%20fakta/Arealer/JO10/JO10SM1002/JO10SM1002_inEnglish.htm (date of access: September 11, 2010)

The Swedish Dairy Association (2010) Milk - key figures Sweden. www.svenskmjolk.se. (date of access: August 17, 2010)

Power Generation from Animal Wastewater Using Microbial Fuel Cell

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Abstract

Microbial Fuel cells (MFCs) are bioreactors, which generate power directly from dissolved organic substrate, such as sugars, organic acids and biomass by using electrogenic organisms as biocatalyst. The concept of MFCs has been known since about 100 years ago, however it hadn't attracted much attention because current and power production was very small. For last 10 years, the current and power production of MFCs has been advanced significantly, and MFCs has attracted attention as a sustainable power production technology. Especially in the field of wastewater treatment, practical application of MFCs is strongly desired because MFCs can achieve both sanitization of water and power generation at the same time. Furthermore, wastewater theoretically contains several times higher potential energy than the required energy for its own treatment. Except MFCs, there already have existed wastewater treatment processes that can achieve energy recovery such as methane fermentation. However, compared to anaerobic digestion providing methane, MFCs have some advantages.

In the field of animal industry, the treatment of large amount of animal manure has been a big problem because it requires a lot of energy. However, this can be reversed, since the animal manure contains great amount of potential energy. The recent increase of anaerobic digester's construction shows the high interest in the energy recovery from this waste.

In this paper, the alternate to this technology MFC, will be discussed.

1. Introduction

From animal industry, large amounts of animal manures are generated every day in the world. In Japan only, 90 million tons of animal manures are generated each year (Ministry of the Environment, 2007).

To prevent environmental pollution, it is important to treat them before release. But it requires much power for treatment. For example, in activated sludge process, which is conventional wastewater treatment process, 1kWh/kg carbohydrate of energy is required (Rabaey and Verstraete, 2005).

However, it is said that wastewater has 9.3 times potential energy (Shizas and Bagley, 2004). In other words, they can be a great energy source. If we can recover and use it for wastewater treatment, total power consumption for wastewater treatment will be reduced, and further, if the amount of recovered energy exceeds the amount of required energy for wastewater treatment, energy can be obtained from wastewater.

From the view of constructing sustainable society, energy recovery from wastewater is quite important.

As an energy recovery system from wastewater, methane fermentation has been already in practical application, in which bio-gas is recovered and used as the fuel for electric generation. However, methane fermentation system has some problems. Bio-gas of methane fermentation includes hydrogen sulfide (Weiland, 2010), which is not only corrosive but also toxic for human.

Microbial fuel cell (MFC) is a novel and alternate to this technology. In this system, electricity is obtained directly instead of recovering as fuel. This

direct power production is achieved by collecting excess reducing power from microorganisms through external circuit.

The concept of MFC was known from about 100 yeas ago (Potter, 1912). But it didn't attract much interest because the power production was very low. But in 1980s, electron mediator was found to enhance power and current production significantly (Roller et al., 1984). Mediators are chemicals, which are reduced by collecting electron from microbes and oxidized by passing electron to the anode. And in 1999, it was reported that some microbes can transfer electron directly to the electrode and they can produce high power and current (Kim et al., 1999). After these breakthroughs, MFC has been regarded as one of the most attractive technology for the energy recovery from wastewater.

As energy recovery process from wastewater, there are many merits of MFC, compared to methane fermentation. First, MFC can produce power directly, so it does not require power generator, whereas methane fermentation requires it. Second, assuming that recovered energy is used as electric power, theoretical energy recovery rate is higher, because it is not restricted by the limitation of Carnot cycle. Third, MFC can be operated lower temperature such as 20˚C, whereas methane fermentation have to be warmed to 37 or 55˚C . Fourth, no need of off-gas treatment and do not need to worry about toxic gas.

In this review, MFC, as a potential technology for energy recovery is presented. Also the possibility of application to animal wastewater treatment is presented.

2. Principal of MFC

MFC is composed of anode and cathode. The schematic diagram of typical two-chamber MFC is shown in Fig.1. In the anaerobic chamber, electrogenic bacteria, such as genus *Geobacter* and genus *Shewanella* degrade organic compounds in the wastewater.

For example, acetate is degraded by following reaction.

$$CH_3COO^- + 2H_2O \rightarrow 2CO_2 + 7H^+ + 8e^-$$

Protons produced by the microbes move to cathode chamber through the ion exchange membrane by diffusion. Electrons also move to cathode through external circuit. On the surface of cathode electrode, electron and proton are reacting to cathode oxidizer. For example, if oxygen is user as cathode oxidizer, water is produced, with following reaction.

$$O_2 + 4e^- + 4H^+ \rightarrow 2H_2O$$

If we set external resistance in the external circuit, potential of electron is converted to power. This is the mechanism of power production in MFC process.

There are two major mechanism of electron transfer from microbes to electrode. One is mediator type, which use some chemicals as electron shuttle. In the mediator type MFC, electrons in the microbes are passed to mediator and mediator become reduced form. On the other hand, mediators pass electrons to electrode and become oxidized form. By being oxidized and reduced repeatedly, mediators play role of electron shuttles from microbes to electrode. The oth-

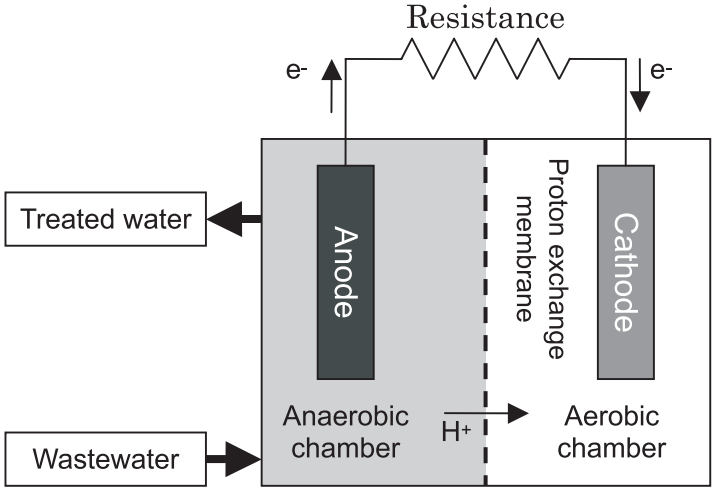

Fig. 1. Schematic diagram of typical two-chamber MFC.

er mechanism is direct electron transportation in the mediator-less MFC. It is thought that microbes make conductive wires called nano-wire and transport electron to electrode through it. But detail mechanism of this direct electron transportation is yet unknown.

3. Classification of MFCs

There are many types of MFC. Here, basic patterns and strategies of MFC for wastewater treatment, are presented.

3-1 Mediator type or mediator-less type

The difference between mediator type and mediator-less type are using mediator or not. The demerit of mediator type is that in case chemical mediator is added, it will flow out with effluent and need continuous addition. To solve this problem, to acclimate mediator producing bacteria in anode chamber is investigated. Anode electrode modification with mediator is also investigated.

Generally, the power production from mediator-less type is higher than mediator type, and is regarded to have advantage.

3-2 Variety of electron acceptor of cathode

As cathode electron accepter, oxygen, ferricyanide and nitrite are used mainly. Ferricyanide cathode can produce highest power of these three. But it is not sustainable, and is not regarded to be applied for practical use (Logan et al., 2006). So, ferricyanide is used as cathode electron accepter when observing anode reaction under it is not restricted by cathode reaction.

Nitrate is sometimes used. The characteristic of nitrate cathode is that nitrate is reduced to nitrogen gas. So with this system, power production and nutrient removal can be achieved at the same time (Clauwaert et al., 2007). However, nitrogen in the organic wastewater is always exists as ammonia nitrogen or organic nitrate, and to convert them to nitrate nitrogen, it require aeration consuming a lot of power. So, the problem of this system is how to prepare nitrate for cathode.

Oxygen is the most major cathode electron acceptor and regarded as electron accepter for future practical application, because there is plenty of oxygen in the air and is sustainable (Logan et al., 2007). To evaluate total performance of the cell, oxygen is used for cathode. However, the reduction rate of oxygen is slow and sometimes cathode reaction becomes limiting factor of the cell, and it make to evaluate limitation of anode difficult. For the MFC using oxygen as cathode electron acceptor, there is a system without anode chamber, called single-chamber MFC. The schematic diagram of this system is shown in Fig.2. In this system, oxygen can directly go through cathode. Since this process does not require aeration, it requires low power for operation. And also, the power production of single-chamber system is higher than two-chamber system (Liu et al., 2004), because the oxygen supply rate is higher compared to two chambered system. So this type is regarded as most probable for practical use.

4. Evaluation methods of MFC

To evaluate capacity of MFC, power production,

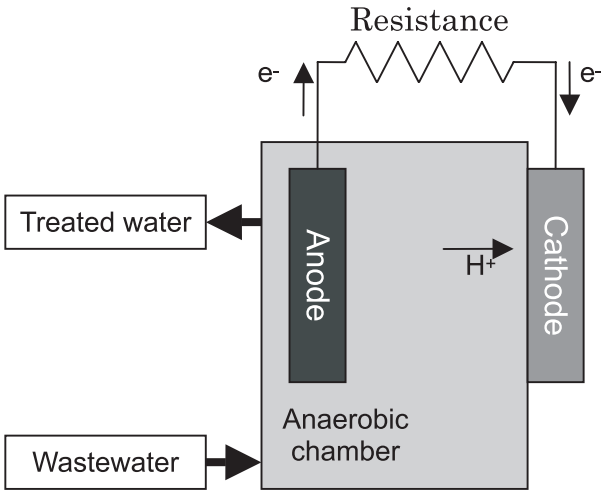

Fig. 2. Schematic diagram of typical single-chamber air-cathodeMFC.

current production, coulombic efficiency, organic removal capacity are measured.

Power production capacity is normally decided based on power density curve, which is a figure plotting relationship of power density and current density Fig.3. These are made by the measuring voltage by changing external resistance. Current, I (A) and power density, P (W/m^2), is calculated according to I= V/R and P = IV/A where V (V) is the voltage, R (Ω) is the resistance, and A (m^2) is surface area. Projected anode surface area of anode is normally chosen as surface area of electrode. The maximum point of power density curve is the maximum power density. Instead of changing external resistance, linear sweep voltammetry technique with potentiostat can be used (Logan et al., 2006). Potentiostat is a device which can control the voltage between two electrodes. Linear sweep voltammetry technique is a technique sweeping voltage two electrode with constant rate and measure current. With this method, relationship between anode and cathode can be obtained. Polarization curve can also be obtained from these data. The slope of polarization curve indicates the internal resistance of MFC (Logan et al., 2006), which affect maximum power production.

Coulombic efficiency (CE) is the value how much proportion of organic substrate is used for current production. Substrate which is not used for current production is consumed by non-electrogenic bacteria or consumed by electorogenic bacteria but used for other metabolism such as growth or internal carbon storage (Freguia et al., 2007). The low coulombic efficiency means the proportion of electrogenic bacteria is low. In highly enriched cell, coulombic efficiency can be as high as 95% (Bond and Lovley, 2003).

The actual charge amount, flown thorough the external circuit can be calculated by the integration of current times second. The theoretical charge amount, when 100% of organic substrate is used for current production can be calculated by the following equation.

$$Q = 96500\ (C/mol) \times COD\ (gO) / 16\ (gO/mol) \times 2$$

CE is calculated by dividing actual charge by the theoretical 100% charge.

Chemical Oxygen demand (COD) is used for concentration of organic substrate. In case of real wastewater, measured value is used. On the other hand, theoretical value can be used when artificial wastewater is used.

Organic removal capacity means how clean the wastewater becomes by the treatment. By dividing the COD of effluent by the COD of influent, this value can be calculated. This value can be changing by the retention time of wastewater.

5. Applications of MFC and similar electrochemical system

When applied to wastewater treatment, MFC and similar bioelectrochemical system can achieve not only power production, but also provide other merit

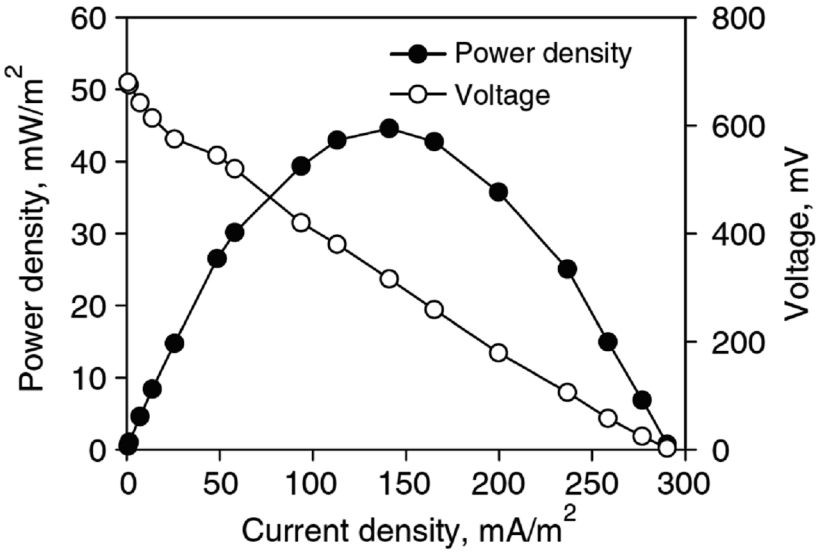

Fig. 3. Polarization and power density curves of a microbial fuel cell operating on swine waste water. The curves are the original data (Min et al., 2005)

such as nitrogen removal, hydrogen and methane production and desalination.

As described before, nitrogen removal can be achieved by using nitrate for cathode of MFC. This system is quite useful, when a lot of nitrate is available.

Hydrogen production is achieved by Microbial electrolysis cell (MEC). In this system, about 0.2V is added from external circuit, by this increase of potential, proton can be reduced to be hydrogen on the cathode. The hydrogen recovery efficiency of this system is higher compared to hydrogen fermentation (Logan et al., 2008). And also, methane is produced with this system (Wang et al., 2009). They reported the rate of methane production is low when higher voltage is applied between anode and cathode.

Microbial desalination cell (MDC) is a process which makes it possible to desalinate water by using MFC mechanism (Mehanna et al., 2010). The configuration of MDC is shown in Fig.4. In anode chamber of the cell, proton is produced and to compensate it, anion such as chloride in the middle desalination chamber goes to the anode chamber through anion exchange membrane. On air cathode, proton is consumed and concentration of OH^- is increase. To compensate it, cation such as sodium in the middle desalination chamber goes to the cathode through cation exchange membrane. As a result of this ion transportation, salinity in the center desalination chamber is achieved.

With the operation of MFC ammonia removal from swine wastewater (Kim et al., 2008) was reported. Ammonia oxidizing bacteria was detected on the cathode, but it cannot be explained only by the nitrification. However, no current production was observed by the addition of ammonium, they concluded that it is caused by ammonia volatilization with the local pH increase near cathode. He et al. (2009) reported that current can be produced by the ammonium in the MFC, but the coulombic efficiency was only 0.3% and almost no contribution for current production. However, it is a fact that ammonia is removed from wastewater and if the technology to collect volatilized ammonia is established, it is can be used for practical application.

In future, along with the progress in research about MFC, much more modification methods, which are new and attractive for wastewater treatment might be developed.

6. Potential of MFC for animal wastewater treatment

High power production by MFC is achieved when wastewater contains, high organic substrate (Liu et al., 2005B), high conductivity (Liu et al., 2005A), and high buffering capacity (Gil et al., 2003). Some animal wastewaters are thought to be suitable for MFC because they meet these conditions.

Table 1. is a comparison of power production from various wastewaters contain artificial wastewater, municipal wastewater, and animal wastewater. It should be noted that these results might be affected by the capacity of each MFCs and it might not be

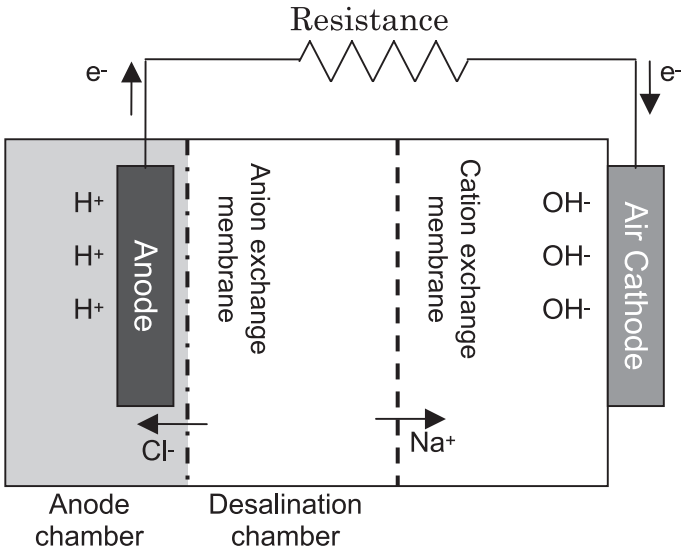

Fig. 4. Schematic diagram of microbial desalination cell

Table 1. Comparison of power production from various wastewaters

Type of substrate	Concentration of organic substrate (mg/L)	Maximum power (mW/m^2)	Coulombic efficiency	Type of MFC	COD removal efficiency	Ref.
Artificial wastewater						
Acetate	1000	2400	41%	Air cathode	-	Logan et al., 2007
Glucose	480 (TOC)	2160	28%	Air cathode	93%	Catal et al., 2008
Real wastewater						
Swine wastewater	8320 (COD)	261	8%	Air cathode	27% (44h)	Min et al., 2005
Farm manure	-	5	-	2 chamber	-	Scott and Murano. 2007
domestic wastewater	200-300 (COD)	146	20%	Air cathode	-	Liu and Logan. 2004
Brewery watewater	2240 (COD)	205	10%	Air cathode	87%	Feng et al., 2008
Meat packing wastewater	6010 (COD)	139	-	Air cathode		Heilman and Logan. 2006
Paper recycling wastewater	1400 (COD)	144	-	Air cathode	29%	Huang and Logan. 2008
starch processing wastewater (diluted to 1/2)	4852 (COD)	239.4	8%	Air cathode	98%	Lu et al., 2009

represent the maximum capacity of each wastewater.

Current production from the substrate of glucose and acetate are higher than real wastewater. It might be because those substrates are easily degradable by the microorganisms. However, real wastewaters are also good fuels for MFC. Furthermore, current production from swine wastewater is higher than other wastewater such as municipal wastewater. That means at least some animal wastewaters are as suitable fuel as or sometimes more suitable than municipal or other wastewaters.

However, there are only few reports about animal wastewater treatment with MFC. Since real wastewater contains misplaced materials and microbes, they might have some inhibitive affect for MFC. So it is important to accumulate knowledge about real animal wastewater treatment by MFC.

MFC is expected as a future energy recovery system from wastewater. There are a lot of animal wastewaters need to be treated and MFC might make it possible to recover energy from them. However, report about animal wastewater for MFC is very few. It is important that not only municipal wastewater, but also animal wastewater treatment with MFC will be investigated.

References

Bond, D. and D. Lovley (2003) Electricity production by *Geobacter sulfurreducens* attached to electrodes. Applied and environmental microbiology, 69: 1548.

Catal, T., K. Li, H. Bermek and H. Liu (2008) Electricity production from twelve monosaccharides using microbial fuel cells. Journal of Power Sources, 175: 196-200.

Clauwaert, P., K. Rabaey, P. Aelterman, L. De Schamphelaire, P. Boeckx, N. Boon and W. Verstraete (2007) Biological denitrification in microbial fuel cells. Environ. Sci. Technol, 41: 3354-3360.

Feng, Y., X. Wang, B. Logan and H. Lee (2008) Brewery wastewater treatment using air-cathode microbial fuel cells. Applied microbiology and biotechnology, 78: 873-880.

Freguia, S., K. Rabaey, Z. Yuan and J. Keller (2007) Electron and carbon balances in microbial fuel cells reveal temporary bacterial storage behavior during electricity generation. Environ. Sci. Technol, 41: 2915-2921.

Gil, G., I. Chang, B. Kim, M. Kim, J. Jang, H. Park and H. Kim (2003) Operational parameters affecting the performance of a mediator-less microbial fuel cell. Biosensors and Bioelectronics, 18: 327-334.

He, Z., J. Kan, Y. Wang, Y. Huang, F. Mansfeld and K. Nealson (2009) Electricity Production Coupled to Ammonium in a Microbial Fuel Cell. Environmental science & technology, 43: 3391-3397.

Heilmann, J. and B. Logan (2006) Production of electricity from proteins using a microbial fuel cell. Water Environment Research, 78: 531-537.

Huang, L. and B. Logan (2008) Electricity generation and treatment of paper recycling wastewater using a microbial fuel cell. Applied microbiology and biotechnology, 80: 349-355.

Kim, B., H. Kim, M. Hyun and D. Park (1999) Direct electrode reaction of Fe (III)-reducing bacterium, *Shewanella putrefaciens*. Journal of Microbiology and Biotechnology, 9: 127-131.

Kim, J., Y. Zuo, J. Regan and B. Logan (2008) Analysis of ammonia loss mechanisms in microbial fuel cells treating animal wastewater. Biotechnology and bioengineering, 99: 1120-1127.

Liu, H. and B. Logan (2004) Electricity generation using an air-cathode single chamber microbial fuel cell in the presence and absence of a proton exchange membrane. Environ. Sci. Technol, 38: 4040-4046.

Liu, H., S. Cheng and B. Logan (2005A) Power generation in fed-batch microbial fuel cells as a function of ionic strength, temperature, and reactor configuration. Environ. Sci. Technol, 39: 5488-5493.

Liu, H., S. Cheng and B. Logan (2005B) Production of electricity from acetate or butyrate using a single-chamber microbial fuel cell. Environ. Sci. Technol, 39: 658-662.

Logan, B., B. Hamelers, R. Rozendal, U. Schröder, J. Keller, S. Freguia, P. Aelterman, W. Verstraete and K. Rabaey (2006) Microbial Fuel Cells: Methodology and Technology†. Environ. Sci. Technol, 40: 5181-5192.

Logan, B., D. Call, S. Cheng, H. Hamelers, T. Sleutels, A. Jeremiasse and R. Rozendal (2008) Microbial electrolysis cells for high yield hydrogen gas production from organic matter. Environmental science & technology, 42: 8630-8640.

Logan, B., S. Cheng, V. Watson and G. Estadt (2007) Graphite fiber brush anodes for increased power production in air-cathode microbial fuel cells. Environ. Sci. Technol, 41: 3341-3346.

Lu, N., S. Zhou, L. Zhuang, J. Zhang and J. Ni (2009) Electricity generation from starch processing wastewater using microbial fuel cell technology. Biochemical Engineering Journal, 43: 246-251.

Mehanna, M., T. Saito, J. Yan, M. Hickner, X. Cao, X. Huang and B. Logan (2010) Using microbial desalination cells to reduce water salinity prior to reverse osmosis. Energy & Environmental Science, 3: 1114-1120.

Min, B., J. Kim, S. Oh, J. Regan and B. Logan (2005) Electricity generation from swine wastewater using microbial fuel cells. Water Research, 39: 4961-4968.

Potter, M. (1911) Electrical effects accompanying the decomposition of organic compounds. Proceedings of the Royal Society of London. Series B, Containing Papers of a Biological Character, 84: 260-276.

Rabaey, K. and W. Verstraete (2005) Microbial fuel cells: novel biotechnology for energy generation. TRENDS in Biotechnology, 23: 291-298.

Roller, S., H. Bennetto, G. Delaney, J. Mason, J. Stirling and C. Thurston (1984) Electron transfer coupling in microbial fuel cells: 1. comparison of redox mediator reduction rates and respiratory rates of bacteria. Journal of Chemical Technology and Biotechnology, 34: 3-12.

Scott, K. and C. Murano (2007) A study of a microbial fuel cell battery using manure sludge waste. Journal of Chemical Technology and Biotechnology, 82: 809-817.

Shizas, I. (2004) Experimental determination of energy content of unknown organics in municipal wastewater streams. Journal of Energy Engineering, 130: 45.

Wang, A., W. Liu, S. Cheng, D. Xing, J. Zhou and B. Logan (2009) Source of methane and methods to control its formation in single chamber microbial

electrolysis cells. International Journal of Hydrogen Energy, 34: 3653-3658.

Weiland, P. (2010) Biogas production: current state and perspectives. Applied microbiology and biotechnology, 85: 849-860.

Web sites

Ministry of the Environment, Current state of discharge and disposal of industrial waste for fiscal year 2007, (2007) (In Japanese; *Sangyouhaikibutsu no haishutsu oyobi shori joukyou tou*) http://www.env.go.jp/recycle/waste/sangyo/sangyo_h19.pdf

Beneficial Effects of Environmental Enrichment and Human Contact with Animals

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Abstract

In animal welfare, we focus on the physical and mental health of the animals with reference to "Five freedoms." The methods of rearing livestock have been studied for animal welfare such as in nutritional studies about feed that satisfies their nutritive requirement (freedom from hunger), studies about environmental resources such as bedding and ventilation (freedom from discomfort), and veterinary studies about preventing injury or disease (freedom from injury or disease). It is also important to investigate the psychological aspects of animals in terms of freedom to express normal behavior and freedom from fear and distress. Environmental enrichment and establishing human-animal bond (for example, brushing) as the methods for improving the psychological aspects of animals in intensive animal husbandry is known. Environmental enrichment is not only for livestock animals and in laboratory animals such as rat and fruit flies. Environmental enrichment and gentle human contact with animals affect the psychological state of animals in a beneficial way, and improve animal welfare.

Introduction

Human beings breed, rear, and nourish livestock as a means of providing themselves with sustenance, i.e. meat, milk, and eggs. To that end, humans supply shelter, food, and other resources for farm animals according to each animal's intended purpose. In the field of animal husbandry, animal welfare is affected by the type of physical environment the animals are housed in, the equipment used upon them, and the way in which they are managed.

When considering the welfare of animals, we focus on both their physical and psychological health in accordance to the '5 freedoms' (Farm Animal Welfare Council 1992), which entitle farm animals to:

1. freedom from hunger and thirst;
2. freedom from discomfort;
3. freedom from pain, injury, or disease;
4. freedom to express normal behaviour; and
5. freedom from fear and distress.

Good psychological health in agricultural animals encourages their biological functions and productivity, benefitting animals and humans alike. To achieve this sense of well-being in farm animals requires enriching their living environment and alleviating any fear they may have of humans. It should be noted, however, that in the case of high-density animal housing, productive labour practices and time-intensive pacification of animals through gentle human contact are in conflict, as are optimal use of unit area and allowing farm animals to express normal behaviour.

Alleviating fear

Livestock often fears humans. This fear affects the animals' welfare and, consequently, their productivity. It has been found that in laying hens, pigs, and dairy cattle, an increase in their fear of humans decreased their productivity (Rushen et al. 1999).

Benign, gentle human contact with an animal can decrease that animal's fear of humans and build a relationship of affiliation between the 2 species. It has been reported that brushing cattle decreases the flight response (Hemsworth and Coleman 1998), with the animals reported to prefer being brushed on the head

(Akasaka et al. 2010) or neck (Schmied et al. 2008).

In contrast, negative handling, such as kicking and beating, increased flight and stress responses, and resulted in less weight gain in cattle (Breuer et al. 2003).

These reports indicate that the type of human contact made with animals determines how an animal responds to humans.

Enriching animal environment

In animal husbandry systems, environmental enrichment is an accepted method for stimulating normal animal behaviour. This has been demonstrated in laying hens (Appleby and Hughes 1995; Shimmura et al. 2007; Shimmura et al. 2010), broiler chickens (Le Van et al. 2000; Kells et al. 2001; Leone and Estevez 2008), pigs (Millet et al. 2005; van de Weerd and Day 2009), and stabled horses (Cooper et al. 2000; Cooper et al. 2005; Mills and Riezebos 2005; Ninomiya et al. 2008). Environmental enrichment also has been studied in beef cattle (Pelley et al. 1995; Wilson et al. 2002; Ishiwata et al. 2006). In artificial pastures, cattle use trees for self-grooming (Kohari et al. 2007), and it has been shown that feedlot cattle use devices for self-grooming (Wilson et al. 2002). Ishiwata et al. (2006) reported that installing a metal barrel (58 cm in diameter, 90 cm in height) containing hay and wrapped in artificial turf in the pen at the early fattening stage stimulated steers' eating and grooming within the pen, and resulted in better muscle characteristics.

We have also studied environmental enrichment for beef cattle previously; we provide here a brief discussion on our findings. In a study conducted in 2009, S. Sato and I enriched the environment in the rearing of Japanese black calves (Ninomiya and Sato, 2009, Fig. 1).

We conducted 3 types of treatments. One treatment was to stimulate the grooming behaviour in calves by setting a brush and a log in their pens. Grooming is recognized as a form of maintenance behaviour in cattle. These animals clean themselves by rubbing their bodies against trees in the pasture (Kohari et al. 2007) or, as in the case of this study, they used the brush and log placed in the pens.

The second treatment involved creating a comfortable resting area for the calves. This was done by removing dirty bedding and manure more frequently, as well as providing new straw bedding more often. This rest area was found to encourage the calves to lie down, another maintenance behaviour in cattle, which conserves their energy, and is conducive to sleep. In the study, sleeping increased in the treatment group of calves compared to that in the control group. Sleeping is known to indicate behavioural satisfaction in animals (Ninomiya et al. 2007), and this treatment succeeded in eliciting this normal behaviour in calves and satisfying this behavioural need.

The third treatment involved the installation of a wooden board in the calves' resting area, as a means of decreasing antagonistic behaviour. A 2-m-wide by 1-m-high wooden wall was placed in the centre of the pen (Fig. 1). If a weak calf was chased by a stronger one, it would pass through the narrow gap (about 75 cm in width) to the other side and hide behind the wall. This would weaken the motivation of the strong calf to chase the weaker one, because the gap was very narrow to pass through. Also, the antagonistic

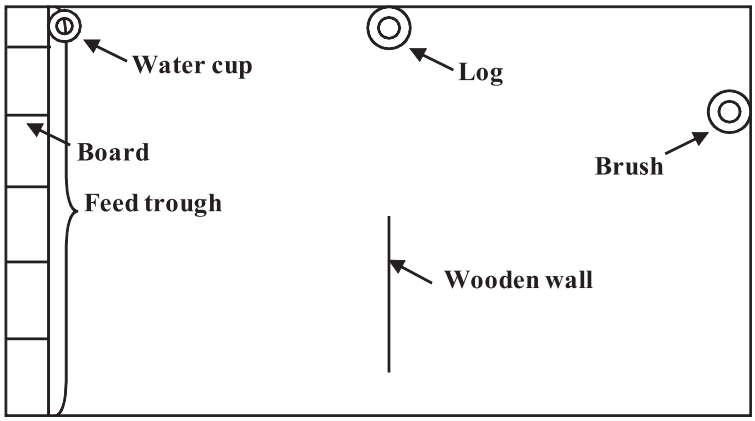

Fig. 1. The environmental enrichment in the pen. Ninomiya, S. and S. Sato (2009). Animal Science Journal 80, 347 - 351.

calf was unable to see the weak calf behind the wall. This system enhances a calf's coping ability in the pen and stimulates the normal behaviour of escaping from a threat.

In all 3 treatments, animals were allowed to express natural behaviours for coping with their environment. Our study suggests that environmental enrichment enhances the animals' expression of natural behaviours and as a result, improves their quality of life.

There is the other aspect of freedom to express normal behaviour, that is, behavioural need (Hughes & Duncan 1988). Many of the behavioural needs of livestock have been established. For example, domestic hens need to build a nest before laying (Hughes et al. 1989), domestic pigs need to root, and cattle need to perform oral manipulation (tongue movement, bite, chew and so on). These behaviours are appetitive and normally precede the act of consumption (feeding, ingesting, drinking). When appetitive behaviour cannot precede consummatory behaviour in the absence of sufficient triggers for appetitive behaviour, animals become frustrated. However, there are many enrichment materials to fulfil these behavioural needs. For domestic chickens, a nest box and perch are used (Appleby and Hughes 1995; Shimmura et al. 2007, 2010, Le Van et al. 2000; Kells et al. 2001; Leone and Estevez 2008). For pigs, pasturing offers opportunities for rooting around in the earth (Millet et al. 2005; van de Weerd and Day 2009).

Environmental enrichment is not a concept to be applied to livestock only. It has also been applied to laboratory animals, resulting in improvements in welfare of those animals (Balcombe 2005). In a number of studies on rats, it has been reported that environmental enrichment (a running wheel, bedding, toys) can improve their ability to learning (Need et al. 2003), enhance their neurogenesis (Ehninger and Kemperman 2003), slow disease progression (Hockly et al. 2002) and suppress the prevalence of stereotypy (Powell et al. 2000). It has also been reported that environmental enrichment improves mating success in fruit flies (Dukas and Mooers 2003). These findings indicate that environmental enrichment encourages normal animal behaviours as well as neuronal function.

Environmental enrichment and gentle human contact with animals affect the psychological state of animals in a beneficial way, and improve animal welfare. These positive effects also serve to encourage an animal's biological functions and correspond to an increase in that animal's productivity. The application of these treatments to intensive housing systems should be a future topic of discussion.

References

Akasaka, C., S. Ninomiya and S. Sato (2010) The effect of brushing by a human on behavioral and physiological stress indicators of cows. The 8th International Symposium on Integrated Field Science (18th–20th September 2010, Sendai), 22.

Appleby, M. C. and B. O. Hughes (1995) The Edinburgh modified cages for laying hens. British Poultry Science, 36: 707-718.

Balcombe, J. P. (2005). Laboratory environments and rodents' behavioural needs: a review. Laboratory Animals, 40: 217-235.

Breuer, K., P. H. Hemsworth and G. J. Coleman (2003) The effect of positive or negative handling on the behavioural and physiological responses of nonlactating heifers. Applied Animal Behaviour Science, 84: 3 - 22.

Cooper, J. J., N. McCall, S. Johnson and H. P. B. Davidson (2005) The short-term effects of increasing meal frequency on stereotypic behaviour of stabled horses. Applied Animal Behaviour Science, 90: 351-364.

Cooper, J. J., L. McDonald and D. S . Mills (2000) The effect of increasing visual horizons on stereotypic weaving: implications for the social housing of stabled horses. Applied Animal Behaviour Science, 69: 67-83.

Dukas, R. and A. O. Mooers (2003) Environmental enrichment improves mating success in fruit flies. Animal Behaviour, 66: 741-749.

Ehninger, D. and G. Kempermann (2003) Regional effects of wheel running and environmental enrichment on cell genesis and microglia proliferation in the adult murine neocortex. Cerebral Cortex, 13: 845-51.

Farm Animal Welfare Council (1992). FAWC updates the five freedoms. Veterinary Record, 131: 357.

Hemsworth, P. H. and G. J. Coleman (1998) Human-Livestock Interactions: The Srockperson and the Productivity and Welfare of Intensively-farmed Animals. CAB International, Oxon, UK.

Hockly, E., P. M. Cordery and B. Woodman (2002) Environmental enrichment slows disease progres-

sion in R6/2 Huntington's disease mice. Annals of Neurology, 51: 235-42.

Hughes, B. O. and I. J. H. Duncan (1988) The notion of ethological 'need', model of motivation and animal welfare. Animal Behaviour, 36: 1696-1707.

Hughes, B. O., A. B. Gilbert and M. F. Brown (1989). The performance of nest building by domestic hens: Is it more important than the construction of a nest? Animal Behaviour, 37: 210-214.

Ishiwata, T., K. Uetake, N. Abe, Y. Eguchi and T. Tanaka (2006). Effects of an environmental enrichment using a drum can on behavioral, physiological and productive characteristics in fattening beef cattle. Animal Science Journal, 77: 352-362.

Kells, A., M. S. Dawkins and M. C. Borja (2001) The effect of a 'freedom food' enrichment on the behaviour of broilers on commercial farms. Animal Welfare, 10(4): 347-56.

Kohari, D., T. Kosako, M. Fukasawa and H. Tsukada (2007) Effect of environmental enrichment by providing trees as rubbing objects in grassland: Grazing cattle need tree-grooming. Animal Science Journal, 78: 413-416.

Leone, E. H. and I. Estevez (2008) Economic and welfare benefits of environmental enrichment for broiler breeders. Poultry Science, 87(1): 14-21.

LeVan, N. F., I. Estevez and W. R. Stricklin (2000) Use of horizontal and angled perches by broiler chickens. Applied Animal Behaviour Science, 65(4): 349-65.

Millet, S., C. P. H. Moons, M. J. Van Oeckel and G. P. J. Janssens (2005) Welfare, performance and meat quality of fattening pigs in alternative housing and management systems: a review. Journal of the Science of Food and Agriculture, 85(5): 709-719.

Mills, D. S. and M. Riezebos (2005) The role of the image of a conspecific in the regulation of stereotypic head movements in the horse. Applied Animal Behaviour Science, 91: 155-165.

Need, A. C., E. E. Irvine and K. P. Giese (2003) Learning and memory impairments in Kv beta 1.1-null mutants are rescued by environmental enrichment or ageing. European Journal of Neuroscience, 18: 1640-1644.

Ninomiya, S., R. Kusunose, Y. Obara and S. Sato (2008) Effect of an open window and conspecifics within view on the welfare of stabled horses, estimated on the basis of positive and negative behavioural indicators. Animal Welfare, 17: 351-354.

Ninomiya, S., S. Sato, R. Kusunose, T. Mitumasu and Y. Obara (2007) A note on a behavioural indicator of satisfaction in stabled horses. Applied Animal Behaviour Science, 106: 184-189.

Ninomiya, S. and S. Sato (2009) Effects of 'Five freedoms' environmental enrichment on the welfare of calves reared indoors. Animal Science Journal, 80: 347-351.

Powell, S. B., H. A. Newman, T. A. McDonald, P. Bugenhagen and M. H. Lewis (2000) Development of spontaneous stereotyped behavior in deer mice: effects of early and late exposure to a more complex environment. Developmental Psychobiology, 37: 100-108.

Pelley, M. C., A. Lirette and T. Tennessen (1995) Observations on the responses of feedlot cattle to attempted environmental enrichment (short communication). Canadian Journal of Animal Science, 75: 631-632.

Rushen, J., A. A. Taylor and A. M. de Passille (1999) Domestic animal's fear of humans and its effect on their welfare. Applied Animal Behaviour Science, 65: 285-303.

Schmied, C., X. Boivin and S. Waiblinger (2008) Stroking different body regions of dairy cows: effects on avoidance and approach behaviour toward humans. Journal of Dairy Science, 91: 596-605.

Shimmura, T., S. Hirahara, Y. Eguchi, K. Uetake and T. Tanaka (2007) Behavior, physiology, performance and physical condition of layers in conventional and large furnished cages in a hot environment. Animal Science Journal, 78(3): 314-322.

Shimmura, T., S. Hirahara, T. Azuma, T. Suzuki, Y. Eguchi, K. Uetake and T. Tanaka (2010) Multifactorial investigation of various housing systems for laying hens. British Poultry Science, 51(1): 31-42.

van de Weerd, H. A. and J. E. L. Day (2009) A review of environmental enrichment for pigs housed in intensive housing systems. Applied Animal Behaviour Science, 116(1): 1-20.

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. Applied Animal Behaviour Science, 76: 259-265.

Diet Selection and Foraging Behavior of Cattle on Species-rich, Japanese Native Grasslands

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Abstract

In addition to serving as livestock pastures, grasslands create wildlife habitats, provide recreational opportunities, and play a key role in watershed regulation. In herbivores, diet selection can profoundly affect the intake of nutrients (e.g., energy, protein and minerals), plant secondary metabolites and toxins. Spatio-temporal heterogeneity in the use of pastures by herbivore livestock means diet selection can also affect vegetational change found on grasslands. In extensive grazing systems, an understanding of this plant-animal interaction is vital for managing animal production and welfare, and ensuring the sustainable use of plant resources. The two goals of this review are to summarize findings regarding diet selection in cattle grazing on Japanese native grasslands and to discuss factors affecting selective grazing of cattle in terms of chemical and behavioral factors. Previous studies showed that Japanese native pastures were composed of 14 to 118 plant species in any given season, of which cattle graze on 22.9–87.5%. Regression analysis showed a strong relationship ($P<0.001$) between the number of plant species in the diet of cattle and the number of species available, with cattle consuming on average 57.9% of the plant species present. Japanese plume-grass (*Miscanthus sinensis*) is one of a particular favorite species, but the underlying mechanisms remain unclear. Among chemical and behavioral factors, bite size significantly related to relative preference (RP) for native plant species. In contrast, both nutrient concentrations and other chemical compounds that affect taste and smell failed to explain RP for native plants. Recent research has shown that the vertical distribution of available leaves is another key factor affecting foraging behavior in cattle. These findings warrant further study of the diet selection mechanism as a complex of chemical-behavioral response.

Introduction

Grasslands are important for grazing livestock. They are also valuable as wildlife habitats, recreational lands, and areas that help regulate watersheds (e.g., WalisDeVries et al., 1998; Willms and Rode, 1998; Shimoda, 2010). While 65% of Japan's land area is covered by forest, grasslands account for a mere 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).

Livestock tend to make heterogeneous use of available land, due to the uneven distribution of available plants, accumulated excreta, soil and water resources, as well as topography (Vallentine, 2000). Grazing animals must forage for and select a diet from a range of available foods to meet nutrient requirements, even in monocultured, intensively managed swards (Forbes, 1995). In species-rich grasslands, animals encounter various plant species whose morphology, availability, quality and phenology differ widely.

The diet selected by herbivores and spatio-temporal differences in land use can have profound implications for nutrient intake (e.g., energy, protein and minerals), the intake of plant secondary metabolites and toxins, and changes in the botanical composition

of grasslands (Vallentine, 2000; Rook and Tallowin 2003; Ogura et al., 2006a). Research on herbivore diet selection and foraging behavior is important for livestock productivity and health and for the biodiversity and sustainability of grassland ecosystems (Fig 1). Despite extensive studies of herbivore grazing designed to deepen our understanding of diet selection in grazing cattle, the underlying mechanisms remain unclear, since so many factors affect selective grazing (Saiga, 1990; Mohammad et al., 1995; Vallentine, 2000).

This review summarizes previous findings on diet selection by grazing cattle on Japanese native grasslands and discusses various factors affecting selective grazing and foraging behavior in cattle, with reference to recent research topics.

1. Number of plant species available and consumed by cattle on Japanese native grasslands

Surveying the plant species that occur in grasslands and that contribute to the diet of cattle is a first step toward understanding diet selection in grazing cattle. Several field measurements have been carried out for different sites, vegetation, animal breeds and seasons (Table 1). The number of plant species available on grasslands ranges from 14 to 118 for each season. This variation may be attributable to the characteristics of the vegetation itself, to differences in sampling methods, or to the number and size of plots or classification of species groups (e.g., *Carex* spp. and *Asteraceae*). Of these plant species, cattle consumed from seven to 55 species, accounting for proportions ranging from 0.229 to 0.875. The researches in Japan and some recent research in Europe (Mayer et al., 2003; Dumont et al., 2007) involved plant canopy measurements (i.e., recording the frequency of grazed/browsed plants) and observations of biting behavior in the animals observed. Microscopic observations of ingesta from esophageal fistula or fecal samples are also known to be useful (Mohammad et al., 1995; Ortega et al., 1995). The number of forage species has grown with the volume of data gathered over the seasons and years. Reviewing past studies of plant selection by cattle in Japanese native grasslands and forests, Okano and Iwamoto (1989) reported that cattle ingested 478 of 644 available plant species. Regression analysis indicates a strong relationship ($P<0.001$) between the number of plant species in the diet of cattle and the number of plant species available (Fig. 2). The value of the slope (0.579) is the average proportion of plant species consumed by cattle in Japanese native grasslands. This linear relationship indicates that cattle consume more plant species when more species are available.

Some reports showed that plant selection among grazing cattle varies from season to season on native grasslands (e.g., Andrew, 1986; Cruz and Ganskopp, 1998; Willms and Rode, 1998). Aizawa et al. (1973) and Fukukawa et al. (1979) reported that cattle consumed a wider range of plant species in autumn than in spring or summer. Fukukawa et al. (1979) also pointed to an increase in the number of plant species consumed by cattle with higher stocking intensities, suggesting that declining forage availability in the autumn expands the number of plant species consumed by cattle.

2. Evaluation of preference of cattle for native plant species

Relative preference (RP) (Hodgson, 1979; Vallentine, 2000), and selectivity index (SI) (Jacobs, 1974; Andrew, 1986; Coates and Penning, 2000) are useful

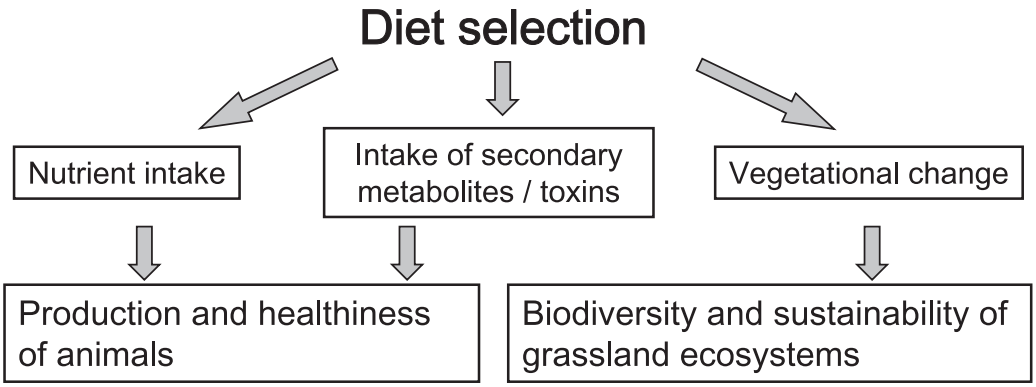

Fig. 1. Importance of diet selection of herbivore animals in grazing systems.

Table 1. A list of researches showing the number of plant species available and consumed by cattle in native grasslands.

Year	Research site	Pasture type	Dominant species	Cattle breed[1)]	Season	No. of species			Measurement technique used for diet estimation	Reference
						as available (A)	as consumed (B)	B/A		
1955	Miyagi, Japan	Native pasture	*Miscanthus sinensis*	JB	summer-autumn	155	76	0.490	Plant based	Iizumi et al. (1956a; 1956b)
1956	Hiroshima, Japan	Native pasture	*Miscanthus sinensis*	JB	spring	14	9	0.643	Plant based	Ito (1962)
1956	Hiroshima, Japan	Native pasture	*Arundinella hirta*	JB	spring	18	15	0.833	Plant based	Ito (1962)
1956	Hiroshima, Japan	Native pasture	*Zoysia japonica*	JB	spring	22	15	0.682	Plant based	Ito (1962)
1956	Hiroshima, Japan	Native pasture	*Plantago asiatica*	JB	spring	16	14	0.875	Plant based	Ito (1962)
1963	Miyagi, Japan	Native pasture	*Miscanthus sinensis*	JB	summer	80	44	0.550	(No information)	Sato (1996)
1969-1971	Ehime, Japan	Young tree plantation	*Miscanthus sinensis*	JB	3 years	185	103	0.557	Plant based	Utsunomiya (1973)
1974	Tochigi, Japan	Native pasture	*Miscanthus sinensis, Cymbopogon tortilis*	HS	autumn	39	22	0.564	Plant based	Fukukawa et al. (1979)
1975	Tochigi, Japan	Native pasture	*Miscanthus sinensis, Cymbopogon tortilis*	HS	autumn	36	12	0.333	Plant based	Fukukawa et al. (1979)
1972	Fukushima, Japan	Native pasture	(No information)	JB	autumn	34	13	0.382	Plant based	Fukukawa et al. (1979)
1973	Fukushima, Japan	Native pasture	(No information)	JB	autumn	26	13	0.500	Plant based	Fukukawa et al. (1979)
1974	Fukushima, Japan	Native pasture	(No information)	JB	spring	34	12	0.353	Plant based	Fukukawa et al. (1979)
1975	Fukushima, Japan	Native pasture	(No information)	JB	spring	26	12	0.462	Plant based	Fukukawa et al. (1979)
1975	Fukushima, Japan	Native pasture	(No information)	JB	autumn	36	22	0.611	Plant based	Fukukawa et al. (1979)
1973	Nagano, Japan	Native pasture	(No information)	AN	autumn	21	13	0.619	Plant based	Fukukawa et al. (1979)
1992	Miyagi, Japan	Native pasture	*Pteridium aquilinum, Miscanthus sinensis*	JB	summer	61	48	0.787	Plant based	Matsumoto and Sugawara (1995)
1992	Miyagi, Japan	Native pasture	*Pteridium aquilinum, Miscanthus sinensis*	JB	autumn	33	23	0.697	Plant based	Matsumoto and Sugawara (1995)
1996-1999	Miyazaki, Japan	Young tree plantation	*Miscanthus sinensis*	JB	4 years	176	118	0.670	Biting behavior	Hirata et al. (2008)
2002	Miyagi, Japan	Native pasture	*Astilbe thunbergii, Miscanthus sinensis*	JB, JS	autumn	49	23	0.469	Biting behavior	Takahashi et al. (2003)
2002	Miyagi, Japan	Native pasture	*Miscanthus sinensis, Calamagrostis arundinacea*	JB, JS	autumn	53	19	0.358	Biting behavior	Takahashi et al. (2003)
2003	Gifu, Japan	Native pasture	*Pleioblastus chino*	JB	spring	87	55	0.632	Biting behavior	Yayota et al. (2008)
2003	Gifu, Japan	Native pasture	*Pleioblastus chino*	JB	summer	102	51	0.500	Biting behavior	Yayota et al. (2008)
2003	Gifu, Japan	Native pasture	*Pleioblastus chino*	JB	autumn	118	55	0.466	Biting behavior	Yayota et al. (2008)
2008	Miyagi, Japan	Native pasture	*Anthoxanthum odoratum*	JB	summer	40	11	0.275	Biting behavior	Yokoyama et al. (2009a)
2008	Miyagi, Japan	Native pasture	*Pteridium aquilinum*	JB	autumn	42	10	0.238	Biting behavior	Yokoyama et al. (2009a)
2008	Miyagi, Japan	Native pasture	*Miscanthus sinensis*	JB	summer	27	7	0.259	Biting behavior	Yokoyama et al. (2009a)
2008	Miyagi, Japan	Native pasture	*Miscanthus sinensis*	JB	autumn	35	8	0.229	Biting behavior	Yokoyama et al. (2009a)
1961	California, USA	Rangeland	*Bromus* spp.	HF	summer	48	42	0.875	Faecal analysis	Van Dyne and Heady (1965)
-	-	-	-	-	-	644	478	0.742	-	Okano and Iwamoto (1989)

1) AN: Angus, HF: Hereford, HS: Holstein, JB: Japaense Black, JS: Japanese Shorthorn.
2) Including number of species and species groups. Each species group was counted as 1 species.

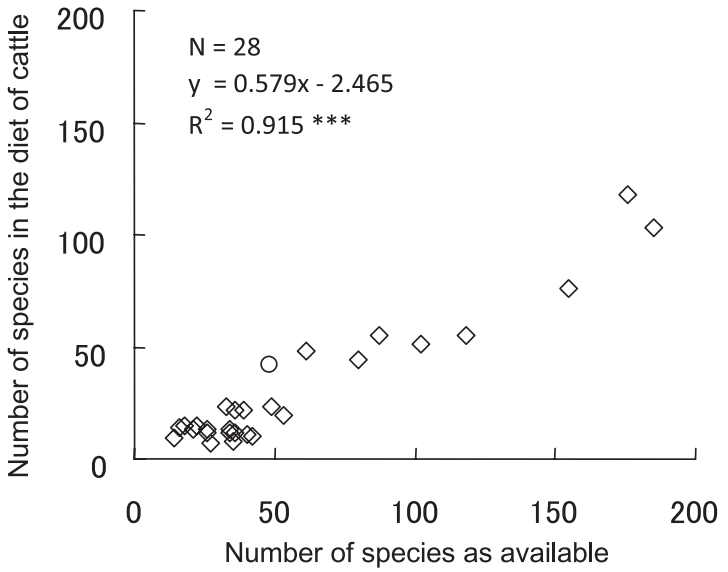

Fig. 2. Relationship between the number of plant species in the diet of cattle and as available in grasslands. The data were collected from Table 1. Diamonds and a circle represent the data from Japan and USA, respectively.

parameters for evaluating how strongly cattle prefer individual plant species. Recent studies showed the high RP of *M. sinensis* among cattle in summer and autumn, both on native grasslands in a northeastern region (Yokoyama et al., 2011) and in a young tree plantation in a southwestern region (Takahashi et al., 2000a; 2000b). The results show cattle graze intensively on *M. sinensis* during the grazing seasons, regardless of climate condition and accompanying plants species.

However, *M. sinensis* is susceptible to damage by defoliation and trampling by grazing cattle and deteriorates under conditions of high stocking intensity within several years (Takahashi et al., 2009). Clarifying why grazing cattle so strongly prefer *M. sinensis* will help improve livestock management and the conservation of native grasslands.

3. Factors affecting plant preference among cattle: chemical and behavioral approach

Grazing researchers have spent considerable time and effort investigating cognitive factors in grazing animals related to forage plants and foraging behavior. Although intake rate maximization theory has generally prevailed in explaining the foraging behavior of large herbivores (Stephens and Krebs, 1986), recent studies suggest the rate of energy intake is not the currency being optimized by the animals (WallisDeVries and Schippers, 1994; Rutter et al., 2004). Current limitations on our understanding in this area make it difficult to extrapolate from simple models to the more complex swards that concern biodiversity managers (Rook and Tallowin, 2003).

In Japanese native grasslands, despite high preference for *M. sinensis*, previous reports point to the relatively low nutritive value of native grasses. For example, concentrations of crude protein (CP) in *M. sinensis* and *Z. japonica* are 69-74 g/kg DM and 71 –102 g/kg DM, respectively, lower than for orchardgrass (*Dactylis glomerata*) (156–171 g/kg DM) growing in the same pasture (Ogura et al., 2001). Hirata et al. (2008) also showed lower dry matter digestibility (DMD; 399–528 g/kg DM) and CP (134–208 g/kg DM) for *M. sinensis* than in the diet of cattle grazing on other species (DMD; 427–587 g/kg DM, CP; 70–150 g/kg DM), due to the higher quality of the major forbs and woody plants consumed by the latter cattle. Takahashi et al. (2006) report on the absence of any significant relationship between intake rates and CP concentrations. Yokoyama et al. (2009b) report that TDN, CP, and minerals failed to explain the RP for native plants. One possible explanation is that toxic plants (*Hydrangea macrophylla* and *Pteridium aquilinum*) have high nutrient concentrations (Yokoyama et al., 2009b).

It is necessary for herbivores to obtain and process information on their foraging choices in a way that maximizes nutritional intake rate and minimizes the risk posed by toxic substances. Several channels are involved in processing this information, including

temporally distinct perspective faculties such as sight and smell, which precede consumption, and retrospective faculties such as touch, taste, gut distention, and nutrient absorption (Illius and Gordon, 1993) (Fig. 3). Botanical and behavioral factors corresponding to these faculties affect the food choice of herbivores in each channel.

3-1. Chemical components affecting diet selection

Saiga (1990) concluded that cattle prefer species with broader leaves, less fiber, fewer toxic substances, more sugar, and higher digestibility. Reviewing the chemical compounds that stimulate the senses of taste and smell, Dohi (1996) classified the chemical components perceived by herbivores as sweet, savory, salty, sour, bitter, or astringent, as gustatory receptors in large herbivores are similar to those in humans. Reports also suggest that the taste sense of livestock is a major factor in the apparent palatability of perennial ryegrass (*Lolium perenne*) (Dohi et al., 1996; 1997b). These studies of gustatory stimuli have been applied to plant breeding. For example, Mayland et al. (2000) reported that concentrations of total soluble sugar correlate with a preference for tall fescue (*Festuca arundinacea*) cultivars. However, information is scarce on the concentrations of chemical components that increase palatability (e.g., soluble sugars and amino acids) in native plants, while a partial finding was reported on the concentration of amino acids and soluble oligosaccharides in *Zoysia species* (Akiyama et al., 1994).

In contrast to gustatory substances, flavor components are less easily classified into the fundamental elements of stimuli like tastes, due to the great variety of perceptions present in olfactory sensations (Dohi, 1996). In addition, the combination of two or more flavor components may result in quite different effects when combined with different olfactory stimuli. Due to the intricacy of the relationship between olfactory sensations and plant flavor components, the chemical components in plants that stimulate olfactory sensation of herbivores have yet to be identified. Thus, most studies have analyzed the volatile components found in foodstuffs (Dohi, 1996).

Researchers have also examined the relationship between leaf surface volatiles found on herbage plants and the corresponding palatability for livestock. Scehovic (1985) reported that volatile compounds reduced the palatability of tall fescue. Dohi et al. (1997a) reported that the presence of cis-3-Hexenol inhibited feeding among goats. Recent research analyzing volatile flavor compounds from samples of the headspace of leaves from four native plants in-

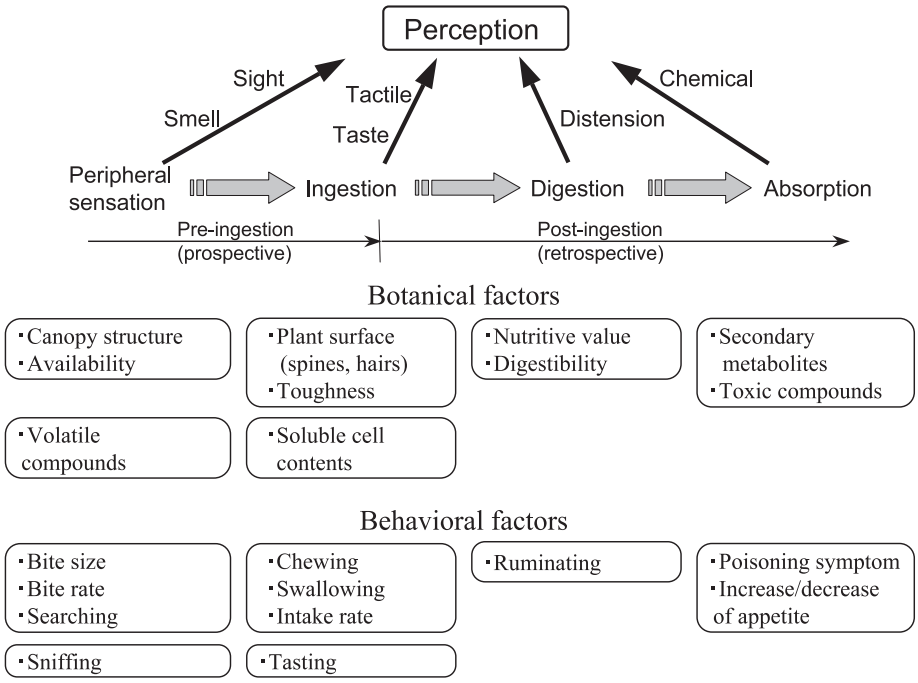

Fig. 3. Flow diagram of the processing of food and information (Illius and Gordon, 1993), and botanical and behavioral elements concerning to each channel of food perception.

dicated that some are unique to certain plant species (Yokoyama et al., 2011). These findings suggest that cattle can use the volatile compounds in association with other plant characteristics, such as the tactile and taste characteristics, as cues for identifying particular plant species.

3-2. Behavioral factors

Behavioral factors interact with the faculties that affect grazing behavior (Fig. 3). Bite size constitutes the smallest and shortest observable component of foraging behavior in space and time, respectively (Senft, 1987; Coleman et al., 1989; Bailey et al., 1996). Takahashi et al. (2006) quantified bite size, bite rate and dry matter intake rate for cattle in a *Miscanthus*-dominated native pasture, suggesting that intake rate of DM and CP could be reduced as cattle took more bites *M. sinensis* due to its smaller bite size. A recent study showed that RP was significantly explained to predicted bite size of beef cattle (Yokoyama et al., 2009a).

Takahashi et al. (2007) examined the relationships between frequency of bites taken by beef cattle and the canopy structure of native plants, focusing on the vertical distribution of biting behavior and plant parts. Their results showed that cows took more bites from layers with more available leaves. For example, for *M. sinensis*, a significant portion of the leaves during summer were found within a height of 40–60 cm, and a high proportion of the bites was distributed within this range of heights. Correlation analysis showed a positive relationship between the proportion of bites and available leaf mass. These results suggest that the distribution of available forage is a major factor of diet selection and foraging behavior in cattle. Ogura et al. (2006b) performed a feeding trial to determine if the height of the available forage affected preference and intake rates for cattle, constructing a structure and setting out equal amounts of hay at different heights. With the hay set at four different heights, cows took more from 70 cm and 120 cm than from the height of 20 cm and 170 cm. The highest intake rate was observed for 70 cm when hay was set at one of the four heights. These findings strongly suggest that vertical height affects food selection and foraging behavior among herbivores. The height of 70 cm is nearly identical to the height at which the leaves of *M. sinensis* abundantly occur (Takahashi et al., 2007). The characteristics of the canopy structure of *Miscanthus*-dominated pastures may offer beneficial foraging conditions that allow cattle to ingest forage plants at higher intake rates.

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

This study examined the selection of native plants as forage by cattle and factors affecting such selection on Japanese native grasslands. Among various chemical and behavioral factors, bite size is a parameter significantly related to RP for native plant species, possibly more so than chemical factors. The distribution of available leaves also proved to be an important factor affecting preference and foraging behavior. These findings help explain why cattle prefer *M. sinensis* and certain other major plants species and should help predict both voluntary intake among herbivores and vegetational change in *Miscanthus*-dominated pastures. However, earlier studies showed that chemical substances derived from native plants could also affect diet selection and foraging behavior. More study is needed to clarify the mechanisms underlying the chemical-behavioral response complex leading to diet selection.

In the next phase of our research, more work will be done to explore the following two questions: Why do grazing herbivores consume such a wide range of plant species? What role does diet selection of herbivores play in the grassland ecosystem? This future study should help advance plant species conservation in native pastures and contribute to productivity gains for grazing livestock.

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References

Aizawa, N., T. Izawa, K. Hayashi, T. Yamagishi and Y. Mizuma (1973) Plant species grazed by Japanese Shorthorn, Japanese Black and Holstein cattle in a native pasture. The Tohoku Journal of Zootechnical Science, 23: 22-23 (In Japanese only. Title translated by present author).

Akiyama, F., H. Sasaki and Y. Saito (1994) Seasonal changes of contents of soluble amino acids and oligosaccharides in turf grasses (*Zoysia*). Bulletin of National Grassland Research Institute, 49: 25-33.

Andrew, M. H. (1986) Selection of plant species by cattle grazing native monsoon tallgrass pasture at Katherine, N. T. Tropical Grasslands, 20: 120-127.

Bailey, D. W., J. Gross, E. A. Laca, L. R. Rittenhouse, M. B. Coughenour, D. M. Swift and P. L. Sims (1996) Mechanisms that result in large herbivore grazing distribution patterns. Journal of Range Management, 49: 386-400.

Coates, D. B. and P. Penning (2000) Measuring animal performance. In: "Field and laboratory methods for grassland and animal production research", Mannetje, L. t' and Jones, R. M. (Eds.), CABI publishing, Wallingford, 353-402.

Coleman, S. W., T. D. A. Forbes and J. W. Stuth (1989) Measurements of the plant-animal interface in grazing research. In: "Grazing research: design, methodology, and analysis", CSSA Special Publication Number 16, G. C. Marten (Ed.), 37-51.

Cruz, R. and D. Ganskopp (1998) Seasonal preferences of steers for prominent northern Great Basin grasses. Journal of Range Management, 51: 557-565.

Dohi, H. (1996) Preference of domestic herbivores for chemical stimuli. Animal Feed Science and Technology, 67: 314-321 (In Japanese only. Title translated by the author).

Dohi, H., A. Yamada and T. Fukukawa (1996) Effects of organic solvent extracts from herbage on feeding behavior in goats. Journal of Chemical Ecology, 22: 425-430.

Dohi, H., A. Yamada, S. Entsu and T. Fukukawa (1997a) Feeding response of goats to leaf alcohol and leaf aldehyde with characteristic leafy flavor. Animal Feed Science and Technology (Jpn.), 68: 474-480.

Dohi, H., A. Yamada and T. Fukukawa (1997b) Intake stimulants in perennial ryegrass (*Lolium perenne* L.) fed to sheep. Journal of Dairy Science, 80: 2083-2086.

Dumont, B., J. P. Garel, C. Ginane, F. Decuq, A. Farruggia, P. Pradel, C. Rigolot and M. Petit (2007) Effect of cattle grazing a species-rich mountain pasture under different stocking rates on the dynamics of diet selection and sward structure. Animal 1: 1042-1052.

Forbes, J. M. (1995) Diet selection. In: Voluntary Food Intake and Diet Selection in Farm Animals. CAB International, Wallingford, 277-303.

Fukukawa, T., R. Igarashi, Y. Kobayashi (1979) Preference of plants by cattle in grazing on pasture in northern Kanto. Bulletin of National Grassland Research Institute, 14: 28-39 (In Japanese with English summary).

Hirata, M., N. Hasegawa, T. Takahashi, R. Chowdappa, S. Ogura, K. Nogami and T. Sonoda (2008) Grazing behaviour, diet selection and feed intake of cattle in a young tree plantation in southern Kyushu, Japan. Tropical Grasslands, 42: 170-180.

Hodgson, J. (1979) Nomenclature and definition in grazing studies. Grass and Forage Science, 34: 11-18.

Iizumi, S., Z. Kurosaki and K. Sugawara (1956a) On the relationship between grazing habits and vegetation of grassland. III. On the grazed plants. The Bulletin of the Institute for Agricultural Research, Tohoku University, 8: 119-124 (In Japanese with English summary).

Iizumi, S., Z. Kurosaki and K. Sugawara (1956b) On relationship between grazing habits and vegetation of grassland. IV. Effect of cattle-grazing and trampling on plant succession. The Bulletin of the Institute for Agricultural Research, Tohoku University, 8: 125-140 (In Japanese with English summary).

Illius, A. W. and I. J. Gordon (1993) Diet selection in mammalian herbivores: constraints and tactics. In: Diet selection: an interdisciplinary approach to foraging behaviour, Hughes, R. N. (Ed.), Blackwell Science Publications, Oxford, 157-181.

Ito, S. (1962) On the grazed wild plants of grassland. Journal of Japanese Grassland Society, 8: 86-90.

Jacobs, J. (1974) Quantitative measurement of food selection. A modification of the forage ratio and Ivelv's electivity index. Oecologia, 14: 413-417.

Matsumoto, H. and K. Sugawara (1995) Herbage intake and identification of plant species fed by grazing animals using plant opal–Identification of plant species grazed by cattle in native grassland using plant opal. Bulletin of Kawatabi Experimental Farm, Faculty of Agriculture, Tohoku University, 11: 33-39 (In Japanese only. Title translated by the author).

Mayer, A. C., V. Stockli, C. Huovinen, W. Konold, B. L. Estermann and M. Kreuzer (2003) herbage selection by cattle on sub-alpine wood pastures. Forest Ecology and Management, 181: 39-50.

Mayland, H. F., S. A. Martin, J. Lee and G. E. Shewmaker (2000) Malate, Citrate, and amino acids in tall fescue cultivars: relationship to animal prefer-

ence. Agronomy Journal, 92: 206-210.

Mohammad, A. G., R. D. Pieper, J. D. Wallace, J. L. Holechek and L. W. Murray (1995) Comparison of fecal analysis and rumen evacuation techniques for sampling diet botanical composition of grazing cattle. Journal of Range Management, 48: 202-205.

Okano, S. and M. Iwamoto (1989) Utilization and palatability of forage plants by livestock in rangeland. Bulletin of Forest and Forest Production Research Institute, 353: 177-211 (In Japanese only).

Ogura, S., T. Kosako, Y. Hayashi and H. Dohi (2001) In *sacco* ruminal degradation characteristics of chemical components in fresh *Zoysia japonica* and *Miscanthus sinensis* growing in Japanese native pasture. Asian-Australasian Journal of Animal Science, 14: 41-47.

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

Ogura, S., T. Shishido and K. Sugawara (2006b) A new technique to evaluate the effects of height of available forage on selectivity and intake rate by cattle. Proceedings of the 40th International Congress of the ISAE, Bristol, UK, 246.

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.

Ortega, I., F. C. Bryant and D. Lynn Drawe (1995) Contrast of esophageal-fistula versus bite-count techniques to determine cattle diets. Journal of Range Management, 48: 498-502.

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

Rutter, S. M., R. J. Orr, N. H. Yarrow and R. A. Champion (2004) Dietary preference of dairy cows grazing ryegrass and white clover. Journal of Dairy Science, 87: 1317-1324.

Saiga, S. (1990) Differences in intake among grass species and cultivars, and factors influencing them. Japanese Journal of Grassland Society 36: 60-66 (In Japanese only. Title translated by the author).

Sato, T. (1996) Natural vegetation of Kitayama grazing land before pasture establishment. Bulletin of Kawatabi Experimental Farm, Faculty of Agriculture, Tohoku University, 12: 105-108 (In Japanese only. Title translated by the author).

Scehovic, J. (1985) Palatability and organoleptic characteristics of the cultivars and hybrids of tall fescue (*Festuca arundinacea* L.). Proceedings of the 15th International Grassland Congress, 317-319.

Senft, R. L., M. B. Coughenour, D. W. Baily, L. R. Rittenhouse, O. E. Sala and D. M. Swift (1987) Large herbivore foraging and ecological hierarchies. BioScience, 37: 789-799.

Shimoda, K. (2010) Ecosystem Service and Grassland Ecosystem. Japanese Journal of Grassland Science, 56: 162-165 (In Japanese only. Title translated by the author).

Stephens, D. W. and J. R. Krebs (1986) Foraging theory. Princeton University Press, New Jersey, 1-215.

Sugiura, T. (2004) Grassland as a wildlife habitat. Grassland Science, 50: 233-238 (In Japanese only. Title translated by the author).

Takahashi, T., M. Hirata, N. Hasegawa, S. Ogura, S. Gondo, K. Nogami and T. Sonoda (2000a) Studies on the interactions between vegetation and grazing cattle in a young *Chamaecyparis obtusa* plantation. 6. Diet selection by Japanese Black cows and dynamics in major selected plant species (1). Grassland Science, 46 (ext.): 352-353 (In Japanese only. Title translated by the author).

Takahashi, T., M. Hirata, N. Hasegawa, S. Ogura, S. Gondo, K. Nogami and T. Sonoda (2000b) Studies on the interactions between vegetation and grazing cattle in a young *Chamaecyparis obtusa* plantation. 7. Diet selection by Japanese Black cows and dynamics in major selected plant species (2). Grassland Science, 46 (ext.): 354-355 (In Japanese only. Title translated by the author).

Takahashi, T., S. Ogura and K. Sugawara (2003) Plant type selection of grazing cattle in sown and native grassland. Bulletin of Integrated Field Science Center, Graduate School of Agricultural Science, Tohoku University, 19: 13–17 (In Japanese only. Title translated by the author).

Takahashi, T., S. Ogura and K. Sugawara, K. (2006) Nutrient intake rate of grazing cattle in a native pasture. Japanese Journal of Grassland Science, 51: 405-407 (In Japanese only. Title translated by the author).

Takahashi, T., S. Ogura and K. Sugawara (2007) Ver-

tical distribution of available foliage and the selectivity of grazing heights by cattle in native grasses. Japanese Journal of Grassland Science, 53: 1-5 (In Japanese with English summary).

Takahashi, T., T. Shishido, S. Ogura and S. Sato (2009) Effects of above-ground damage due to cattle grazing on the survival and regrowth of *Miscanthus sinensis* tillers. Japanese Journal of Grassland Science, 55: 15-20.

Utsunomiya, T. (1973) The palatability and contribution of native plant species to the forage of beef cattle in the case of forest grazing. Journal of Japanese Grassland Science, 19: 154-160. (In Japanese with English summary).

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

Van Dyne, G. M. and H. F. Heady (1965) Botanical composition of sheep and cattle diets on a mature annual range. Hilgardia, 36: 465-492.

WallisDevries, M. F. and P. Schippers (1994) Foraging in a landscape mosaic: selection for energy and minerals in free-ranging cattle. Oecologia, 100: 107-117.

Wallis De Vries, M. F., J. P. Bakker and S. E. Van Wieren (1998) Grazing and conservation management. Kluwer Academic Publishers, Dordrecht, Netherland, 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.

Yayota, M., M. Nakano, J. Karashima and S. Ohtani (2008) Forage selection by beef cows grazed on a dwarf bamboo (*Pleioblastus argenteostriatus* f. glaber)-dominant pasture. Grassland Science, 54: 33-39.

Yokoyama, M., M. Hori, S. Ogura (2009a) Foraging mechanisms of large herbivores in species-rich vegetation. 1. Evaluation of plant selectivity by corresponding to foraging behavior with available biomass in a native pasture. Japanese Journal of Grassland Science, 55 (ext.): 107 (In Japanese only. Title translated by the author).

Yokoyama, M., M. Hori and S. Ogura (2009b) Foraging mechanisms of large herbivores in species-rich vegetation. 2. Relationships of bite size, nutrient concentration and volatile components to plant selectivity. Japanese Journal of Grassland Science, 55 (ext.): 107 (In Japanese only. Title translated by the author).

Yokoyama, M., S. Tanaka, M. Hori and S. Ogura (2011) Factors affecting plant selection of grazing cattle in a diverse, native grassland in Japan. Proceedings of 9th International Rangeland Congress, Rosario, Argentina (in press).

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On-Farm Assessment of Animal Welfare in Japanese Dairy Cattle

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Keywords: animal welfare, cow comfort, on-farm welfare assessment, Animal Needs Index (ANI), certification system, dairy cows

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Abstract

Rapid progress has been made recently toward the establishment of international guidelines related to animal welfare. A similar trend is needed in Japan to prepare methods for evaluation and assessment of animal welfare in terms of scientific judgment of animal welfare at sites of animal husbandry. The authors attempted to prepare a Japanese-style method for the assessment of animal welfare based on modified ANI, which is an improved ANI, and based on the Five Freedoms. Although producers and consumers in Japan have been little interested to date, it is considered that the development of a system to certify animal welfare is needed. It must be beneficial to both producers and consumers.

Introduction

In recent years, concern related to animal welfare has increased worldwide, not just for pets but for animals of all kinds, including farm animals. Although the concern expressed by Japanese consumers and producers remains low, scientific research is necessary to prepare for rising future concerns that are expected.

What is animal welfare?

The Farm Animal Welfare Council established in 1979 by the British government proposed the Five Freedoms shown below in 1993. They have since become the current international consensus:

Freedom from hunger and thirst
Freedom from discomfort
Freedom from pain, injury and disease
Freedom to express normal behaviour
Freedom from fear and distress

The maximum satisfaction of these freedoms engenders improved animal welfare. It is presumed to be vitally important to comprehend “how animals feel” scientifically.

International movement in OIE and EU

The International Epizootic Office (OIE) is a UN agency with 176 member countries including Japan; it has prepared standards for animal diseases and quarantine. In 2005, it also established animal welfare guidelines concerning the transport of animals by land, the transport of animals by sea, the transport of animals by air, the slaughter of animals for human consumption, and the killing of animals for disease control purposes. Additionally, guidelines for rearing management have been scheduled. Such an international movement is destined to impact livestock industry throughout the world.

In the EU, the Welfare Quality (WQ) project was started in 2004–2009. Studies were conducted with the intention of establishing livestock product brands of the highest quality with consideration to animal welfare. Seventeen million euros went into the project for research and development expenses for education, technology, an assessment and certification system, etc. In 2009, the WQ animal welfare assessment method was released for poultry, pigs, and cattle. International promotion of dairy and meat products and eggs with the WQ label is planned in the future to signify a brand of products that certifies animal welfare.

Strict legal restrictions exist in the EU. It has been determined that the battery cage, which has been commonly used as the intensive husbandry system for egg layer chickens, shall be banned by 2012, and

the tethering of sows has already been banned. Moreover, supermarkets and animal protection groups have independently prepared standards for animal welfare assessment to be displayed on labels on the products that meet the standards. It has therefore become a major purchasing motive that a product has been produced considering animal welfare.

Movement in Japan

The Shinshu Comfort Livestock Farm Certification Standard was released in 2007. It included sustainable livestock farming proposed by the Matsumoto Livestock Hygiene Service Center, Nagano Prefecture, and included rearing standards considering animal welfare. Standards for the assessment of animal welfare were prepared respectively for dairy cattle, beef cattle, pigs, laying hens, and broiler chickens; simultaneously, the Ministry of Agriculture, Forestry and Fisheries of Japan finally took action. Its contractor, the Japan Livestock Technology Association, has been holding review meetings related to rearing management of farm animals corresponding to animal welfare. Section meetings are also held by farm animal species under the promotion committee comprising academic experts, producers, etc., along with the progressive formulation of rearing management guidelines. Guidelines for laying hens and pigs prepared in 2008 have already been released. Rearing management guidelines for broiler chickens and dairy cattle are to be released in 2009, and those for beef cattle and horses in 2010. The guidelines are not very strict compared to the EU standards. It is expected that rearing management according to the guidelines shall be required in the future.

What is rearing management of farm animals according to animal welfare? The On-farm assessment method of animal welfare objectively and specifically identifies problems of animal welfare and the specific points for producers to take note of.

Methods for on-farm assessment of animal welfare

(1) Assessment methods in Germany and Austria

The method was released in 1985 by Bartussek as Tiergerechtheit (TGI). It was translated into English and published as the Animal Needs Index (ANI) in 1991. Two types of ANI exist: ANI200 used in Germany and ANI35L used in Austria (Bartussek 1999).

Those ANI were respectively prepared for cattle, sows, and laying hens. In fact, ANI35L has been used since 1995 as the official assessment indicator for organic husbandry in Austria, whereas ANI200 has been used as a recommendation tool for organic farmers in Germany for improvement of the welfare level. The ANI35L/2000-cattle standard, which is ANI for cattle, has been translated into Japanese by the authors (Seo and Kohari, 2006ab; Kohari and Seo, 2006). It scores the rearing environment of farm animals according to five categories of "Locomotion," "Social interaction," "Flooring," "Light and Air," and "Stockmanship." The welfare level is as higher if the score is higher. The assessment is also simple to execute. It can be completed in a short time: about two hours.

The authors (Seo et al., 2007) assessed the welfare level of dairy farms in Hokkaido using ANI for cattle. Subjects were 25 farms in Tokachi Subprefecture with 36 dairy herds: 1) free stalls with outdoor areas (4 herds of 2 farms); 2) free stalls without outdoor areas (12 herds of 8 farms); 3) tethering with outdoor areas (4 herds of 4 farms); and 4) tethering without outdoor areas (16 herds of 11 farms). Fig. 1 shows the ANI scores of all subject farms. The mean was 17.8, the maximum was 40.0, and the minimum was 6.0. The top 4 herds were held in free stalls with outdoor areas, although the bottom 13 herds were held with tethering without outdoor areas. Consequently, the most important matter is to rear cattle in free stalls with outdoor areas to enhance the welfare level assessed by the ANI score.

As Kohari et al. (2006) pointed out, the welfare level secured by this method is determined almost entirely by the facility assessment such as the presence of outdoor areas and free stalls or tethering. Although outdoor exercise is in fact important for dairy cows, most rearing environments have no outdoor areas in Japan. It is difficult to install new pastures or paddocks. It is not desired that the assessment result be low only because of the rearing method even if the housing environment is appropriate.

(2) Assessment methods of RSPCA and others

In Europe and in the US, the rearing standards are defined by animal protection groups and producer groups. First, the most well known assessment method of the Royal Society for the Prevention of Cruelty to Animals (RSPCA) of Britain is introduced here.

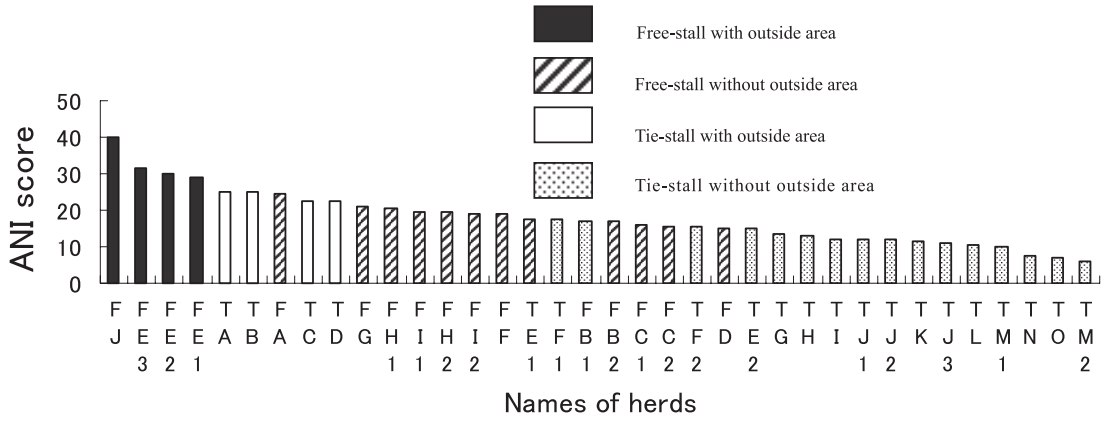

Fig. 1. ANI scores of all herds

The RSPCA is the oldest animal protection group in Britain. It developed the food label called Freedom Food in 1994 which shows consumers if the product is produced in consideration of animal welfare. The assessment standards were prepared assuming that they should meet the Five Freedoms, and if a product meets the standards, the product can be sold with this label displayed on it. There are checkups not only of rearing management but also transport and slaughter with frequency of about once a year.

There are currently standards for chickens, turkeys, ducks, pigs, dairy cattle, beef cattle, sheep, and framed salmon. As many as nine hundred million animals related to livestock industry are produced annually in farms with this certification. Moreover, it is necessary to be well informed on the Code of Recommendations for the Welfare of Livestock prepared by the Department for Environment, Food and Rural Affairs (DEFRA).

In the US, there are also assessment standards resembling those of the RSPCA. A nonprofit organization Humane Farm Animal Care defines animal welfare rearing standards, and certifies that if a product meets the assessment standards to allow labeling on the product. Currently, standards exist for beef cattle, broiler chickens, laying hens, dairy cattle, goats, pigs, sheep, turkeys, and young dairy beef.

Animal welfare assessment methods in Japan

(1) Improved ANI

The authors (Seo et al., 2008) prepared improved ANI in which several problems of the ANI were modified. The improved ANI assigns lower scores to outdoor areas such as pastures and paddocks to avoid higher scores because of them, and additionally includes assessment parameters related to stockmanship, such as a body condition score, so that higher scores are obtainable through the consideration of stockpersons. Using these, dairy farms in Tokachi Subprefecture in Hokkaido makes assessments with this improved ANI. The relation between the numerical welfare level (higher scores indicate higher welfare levels) and data of livestock mutual aid and the milk performance test were demonstrated.

Results from 19 herds of 14 dairy farms by the tethering system are presented in Table 1. Significant negative correlation was found with the percentage of deselected cows caused by diseases (r= -0.45), the death/disuse part of mutual aid premium per cow (r= -0.50) and the total burden on farm households per cow (r= -0.46) on a management basis; and with the percentage of deselected cows caused by diseases (r= -0.70), the death/disuse part of the mutual aid premium per cow (r= -0.45), the percentage of animals with conflict/abnormal behaviours (r= -0.70), the percentage of discontinued lying-down movement (r= -0.72), and the frequency of discontinued grooming (r= -0.77) on an animal basis, respectively. Similar results were obtained at farms using the free stall system (data not shown). These results suggest that consideration of animal welfare can provide expected economic benefits accompanying the reduction of diseases and that they are also desirable for cattle behaviours.

This method, however, is an assessment based on ANI; the problems described earlier are not solved completely; we recognized the need for reconstruction based on the Five Freedoms and prepared the Japanese-style animal welfare assessment method as shown in the next section.

Table 1. Correlation coefficients between welfare score and basis in tethering system.

Basis	Facility	Management	Animal
Production (milk performance test)			
Percentage of deselected cows because of disease	-0.29	-0.45**	-0.70**
Mean linear score	-0.28^	-0.12	-0.64^
Health (mutual aid premium)			
Death/disuse part of mutual aid premium per cow	-0.22	-0.50*	-0.45*
Total burden on farm households per cow	-0.13	-0.46*	-0.41
Behaviour (behavioural observation)			
Percentage of animals with abnormal behaviours	-0.55^	-0.31	-0.70*
Percentage of discontinued lying-down movement	-0.36	-0.34	-0.72*
Frequency of discontinued grooming	-0.47	-0.50^	-0.77**
Duration of rumination while standing	-0.51^	-0.28	-0.51^

*P<0.05, **P<0.01, ^P<0.1

(2) Japanese-style animal welfare assessment

The authors have been approaching the preparation of animal welfare standards related to the rearing management of cows from the following viewpoints: objectivity of standards centered on the Five Freedoms so that stockpersons can conduct a self-check; the assessment can be completed quickly; the assessment is not a simple evaluation of facilities and equipment; and the assessment should also adopt opinions of consumers and producers and win international recognition in the future.

In addition, the actual situation of most farms in Japan is that they have no large land area and that they purchase most feed; grazing is possible only in a few areas such as Hokkaido. Consequently, assigning a higher final score was avoided for grazing itself only, irrespective of the cattle housing environment.

The assessment method we prepared consists of three bases of facility, management, and animal and includes 54 parameters in all. The assessment parameters are listed below:

Facility basis: Feed banks, Heat stress, Air quality, Cow trainers, Condition of passages, Number of stalls per head, Feed space per head, Illumination, Dimensions of rest areas, Passage width, Foot baths for persons, Outdoor areas, Dimensions of water troughs, Water flow, Noise, Tethering method, Crossing passages, Calving place, Blind-alleys, cow brushes

Management basis: Cleanliness of feed banks, Water supply to calves, Slipperiness of beds, Dehorning, Handling tools, Cleanliness of water trough, Weaning period, Cleanliness of beds, Accessory teats, Washing of nursing tools, Stray voltage, Failure of facilities, Number of times of hoof trimming, Cow handling, Nursing of calves, Softness of beds, Tail docking, Downer cows, Colostrum supply, Tethering of calves, Social behaviour of calves, Grouping of calves, Feed supply to calves

Animal Basis: BCS, Conditions of hocks, Injuries, Conflict and abnormal behaviour, Standing up behaviour, Twisted tails, Cleanliness of bodies, Condition of hooves, Flight response, Skin disease, Number of heads suffering from diseases, injuries, and accidents, Number of heads of death/disuse accident

Assessment criteria that are as objective as possible are defined for the parameters presented above based on a literature search. Parameters are to be checked as yes/no if the criteria are met. Although the time needed for the assessment depends on the herd size, the assessment will generally be completed in 2–3 hours.

It is planned to assess the welfare level of cows in Japanese dairy farms using this assessment method in future.

Recognition of animal welfare by consumers

The authors conducted a questionnaire survey related to animal welfare with 600 customers who visited a supermarket in Obihiro, Hokkaido, in 2008. The recovery rate was 80.5%.

Subjects answered questions after receiving a simple explanation of the concept of animal welfare. The item "I want to buy milk associated with animal welfare." was answered yes by 90.4% and no by 9.6% or respondents, indicating that a vast majority wanted to buy the milk. In addition, the median of the mean willingness to pay (MWTP) was +37 yen of the usual purchase price of milk in subjects who answered yes. The reasons for wanting to buy milk associated with animal welfare were considered to include "safety," "to support dairy farmers," and "the cows are apparently healthier" in descending order. The concept of animal welfare has been little known to general consumers; the provision of accurate information is apparently necessary.

Moreover, a possible influence of the experience of farm visit on interest in animal welfare was noted (Table 2). More subjects who had visited a farm tended to be "very interested" in animal welfare than those without such an experience ($\chi 2=4.6$, $P=0.1$). We think that it is most effective for consumers to hear from producers directly and to have actual experience of agriculture at production sites such as educational farms to increase the number and awareness of consumers who understand and support animal welfare.

Table 2. Relation between consumer visits to production fields and consumers' interest level.

Level of interest in animal welfare	Experience of farm visit?	
(Number of people)	No	Yes
Very	49	98
Interested to some degree	121	154
No interest	7	9

Investigation conducted in supermarkets in Obihiro city in 2009.

Recognition of animal welfare by producers

In the questionnaire survey conducted by the authors at 27 dairy farms in Tokachi, Hokkaido, in 2005 (Table 3), 70.4% had not heard of animal welfare and 37.0% had not heard of animal comfort, indicating that cow comfort is better known than animal welfare among dairy farmers.

There are not many producers who can candidly accept animal welfare, which is accompanied by very strong impressions of a required increase in production costs, work time, and work loads, as well as facility improvement, and of reduced productivity. Actually, the cost burden in the aspect of facilities to a certain degree might be unavoidable to aim for improved welfare. However, as described above, it is necessary to inform producers of the possible economic benefits that can be derived from improved animal welfare.

Table 3. Recognition of animal welfare and animal comfort for dairy farmers.

	Animal welfare	Cow comfort
Have not heard of it before (%)	70.4	37.0
Have heard of it before (%)	7.4	18.5
Very important (%)	22.2	44.4

27 dairy farms in Tokachi, Hokkaido were investigated in 2005.

Construction of the animal welfare certification system

Primarily, it is necessary to win the understanding of producers of the concept of animal welfare. We believe that this assessment method is useful for that purpose. It is also important to provide information related to benefits that might be derived from consideration of animal welfare. Moreover, we believe that a system must be made that joins producers and consumers who seek to produce and consume such products. Although direct delivery from producing areas and direct sale in which producers and consumers are directly connected are effective, it is difficult to conduct these at many dairy farms. We suggest that it is necessary to develop a system by which products are certified and distributed as products that consider animal welfare. However, we also think that it is important not to import animal welfare assessment methods of Europe and the US directly but to construct a certification system based on the development of an original, Japanese-style assessment method.

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References

Bartussek, H. (1999) A review of the animal needs index (ANI) for the assessment of animal's well-being in the housing systems for Austrian proprietary products and legislation. Livestock Production Science, 61: 179-192.

Kohari, D., T. Kosako, M. Fukasawa, H. Tsukada and S. Sato (2006) Animal welfare evaluation of grazing environment by animal needs index: ANI35L/2000-cattle (case study). Animal Behaviour and Management, 42(2): 93-100.

Kohari, D. and T. Seo (2006) The dawn of animal welfare and on-farm assessment (2) ANI-35L/2000 for cattle [2]. Sustainable Livestock Production and Humane Welfare, 60(4): 457-462 (in Japanese).

Seo T. and D. Kohari (2006a) The dawn of animal welfare and on-farm assessment (2) ANI-35L/2000 for cattle [1]. Sustainable Livestock Production and Humane Welfare, 60(3): 353-355 (in Japanese).

Seo T. and D. Kohari (2006b) The dawn of animal welfare and on-farm assessment (2) ANI-35L/2000 for cattle [3]. Sustainable Livestock Production and Humane Welfare, 60(5): 571-578 (in Japanese).

Seo, T, K. Date, T. Daigo, F. Kashiwamura and S.Sato (2007) Welfare assessment on Japanese dairy farms using the Animal Needs Index. Animal Welfare, 16(2): 221-223.

Seo, T., Y. Ono, F. Kashiwamura and S.Sato (2008) Improved Animal Needs Index (ANI) related to milk production and cow health on Japanese dairy farms. 4th International Workshop on the Assessment of Animal Welfare at Farm and Group level, 120.

Animal Wastewater Treatment Using Constructed Wetland

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Abstract

In this study, constructed wetlands(CWs) hybridized with vertical and horizontal flow systems treat wastewater from milking parlor. Water quality, the number of coliform bacteria and ammonia oxidizing microbes during the treatments were investigated. In the results, rate of removal COD, TN and TP from raw wastewater were more than 90% from June to September. However, these rates were decreased after October, the lowest removal was observed in December. At this time, ammonia oxidizing bacteria were $10^2 \sim 10^4$ cells/cm^3 by analysis of realtime PCR. Ammonia oxidizing archaea also found 10^2 cells/cm^3. However, ANAMMOX bacteria were not detected in December. The removal of coliform bacteria was not different from September to December, except on a single day. Furthermore, no significant differences in treatment efficiency among the three types of wetlands, were observed. In particular, the no difference in the removal of coliform bacteria by wetlands, constructed with or without plants was found. The highest removal efficiency of coliform bacteria was observed on October 22, which followed 3 days of sunny weather. This observation suggests that soil-drying due to the absence of influent was important for removing coliform bacteria.

To reuse treated water for agricultural water, concentrations of COD and TN are required under 6 mg/L and 1 mg/L respectively. In addition, reclaimed water for landscape requires that the number of coliform bacteria is under 1000 cfu/100ml. The concentration of COD, TN and coliform bacteria in the treated water in summer met the standard to reuse for agricultural water or landscape water.

In the future, more improvement of removal efficiency with the constructed wetland is required for reuse animal wastewater. In addition, it has to promote to establish the recycling system including utilization of treated water.

1. Introduction

In the world, animal meat, milk and eggs were produced 873 million ton (FAOSTAT,2007). Amount of these products was trend to increase. Runoff from animal farms where excessive nutrients are generated has been linked to downstream eutrophication of surface waters. In Japan, there are more than 100 million livestock farmers that have to observe the wastewater treatment standard in Japan.

Table 1 shows wastewater quality from milking parlor. COD concentrations were around 1000 to 2500 mg/L, SS were more than 600 mg/L. TN concentrations were also high, 163-1139 mg/L. TP concentrations were 65.7 -136.3 mg/L. Comparing wastewater from milking parlor with national effluent standard in Japan, all items should be removed. Not only chemicals, but also coliform bacteria in wastewater should be decreased under 3000 cells/ml.

Activated sludge method is popular system for animal wastewater treatment. To activate aerobic microorganisms, aeration was done. This aeration use energy about 56% of all energy required for wastewater treatment. Wastewater treatment using activated sludge from the farm, amount of wastewater was

Table 1. Wastewater quality from milking parlor

COD	1163 ~ 2586	mg/L
BOD	1383 ~ 2476	mg/L
SS	670 ~ 5833	mg/L
Coliform bacteria	134 ~ 6200	cells/mL
TN	163 ~ 1139	mg/L
TP	65.7 ~ 136.3	mg/L
pH	5.8 ~ 7.2	

Yoshio et al.(2004), Sato et al.(2005)

about 3 ton/day, cost of electricity for aeration was about 13000 yen/month, and CO_2 emission by aeration was 79 kgC/month. To achieve the Kyoto Protocol, we should reduce CO_2 emission from wastewater treatment. That reason, constructed wetland received attention.

Constructed wetland was wastewater treatment system using natural purification. It had several advantages, energy-saving, less maintenance, low cost, natural landscape and biodiverse habitats. The first constructed wetland; full scale of FWS (Free water surface CW) was started in Netherlands in the late 1960s. There are various constructed wetlands in the world. Constructed wetlands treat various wastewater, sewage, mining water, landfill leachate, industrial effluent, surface run-off, agricultural run-off, and road run-off.

There are some reports about animal wastewater treatment with CW. Hammer(1992) reported marsh-pond-meadow wetland. This system consists 3 steps. This system achieved to remove the 71% of ammonia. United states Department of Agriculture, Natural Resources Conservation Service(NRCS, 1991, 1992) recommended subsurface flow CW. Vegetation was commonly selected Giant reed in Europe, and NRCS.

Ammonia oxidation is critical to grobal nitrogen cycling and is ofen though to be driven only by ammonium-oxidizing bacteria. The recent finding of new ammonia-oxidizing organisms belonging to the archaeal domain challenges this perception. Two major microbial groups are now believed to be involved in ammonia oxidation: chemollithotrophic ammonia-oxidizing bacteria(AOB) and ammonia-oxidizing archaer(AOA).

Removal of coliform bacteria using constructed wetlands was the one of assignments for reuse of water. There were some reports that removal of indicator organisms with constructed wetlands. However the mechanisms for disappearance of coliform bacteria is not clear enough. This study, monitoring the number of coliform bacteria in the constructed wetland system, was intended to clarify the factors leading to the decrease of coliform bacteria.

In this study, constructed wetland Kawatabi was hybridized with vertical and horizontal flow systems to treat wastewater from a milking parlor. To reuse animal wastewater by treatment with constructed weteland, water quality of treated water using constructed wetland was investigated during treatment. Especially, we investigated about ammonia oxidizing microbes' presents, and the number of coliform bacteria.

2.Material and method

2.1 Study site

The systems were located in Miyagi prefecture, Japan. It snows in winter. They consisted of three types of five-stage CWs for dairy wastewater (Fig.1). The area was 111 m^3, and the depth was 70 cm. The beds were consists of sand and gravel. The first to fourth stages were operated vertical flow bed, the fifth stage was horizontal flow bed. Type A and C were planted with *Phragmites*, type B was not. Water levels of Type A and B were 0 cm, that of Type C was 35 cm (Fig. 2).

2.2 Wastewater and operating

Wastewater was flow from the dairy farming into the CWs twice a day. The volume of wastewater was about 2 m^3 per day. Wastewater quality was shown in Table 1.

The operated conditions adopted the rotation method that was cycled the inflow period and the stop period. The inflow period was for four days from three days, the stop period were for 10 days.

Wastewater and treated water were sampled from September to December every week. Then the sample was measured water qualities, COD, BOD, TN, TP, NH_4-N, NO_2-N, NO_3-N.

2.3 Soil sampling and DNA extraction

The sand and gravels were collected from below the surface to approximately 20 cm when the inflow period was started, in December 2009. Three samples were collected from each condition. From 2 g to 15

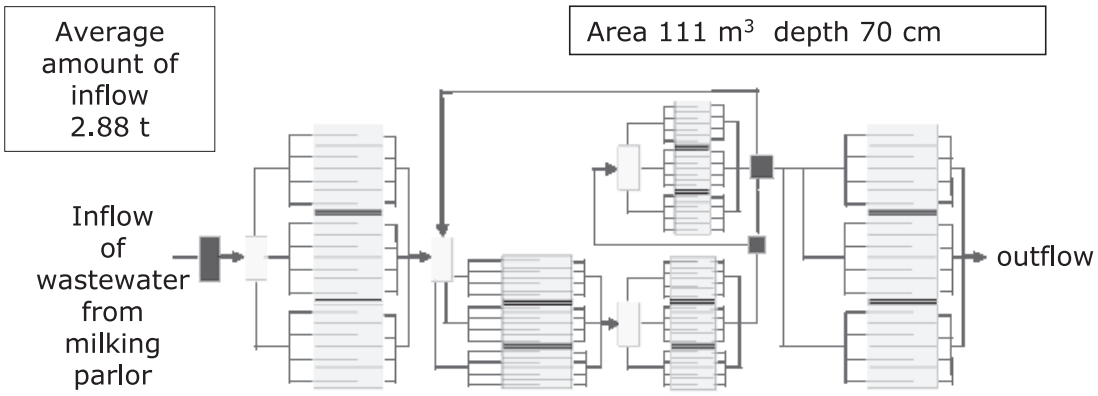

Fig. 1. Schematic of constructed wetland in Kawatabi. Hybridized with vertical and horizontal flow systems to treat wastewater from a milking parlor.

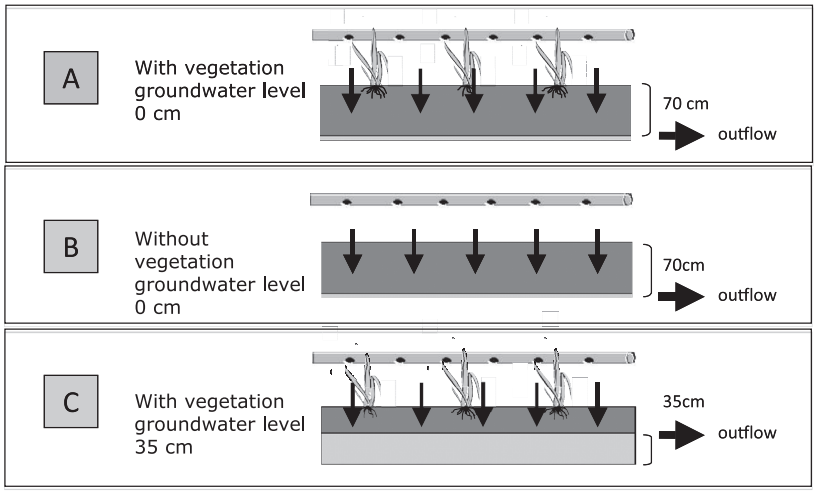

Fig. 2. 3 Types of the constructed wetland in Kawatabi. A has vegetation, and the groundwater level is 0 cm. B has no vegetation, and the ground water level is 0 cm. C has vegetation, and the groundwater level was 35 cm.

g of sand or gravels were shaken with PBS buffer for 10 mins using voltex mixure, after that suspending solution was transfer to a centrifuged tube. The solution was centrifuged by 15000 rpm for 5 mins, the precipitation was used for DNA extraction. DNA from the sand and gravels were extracted using Power Soil Extraction kit (MOBIO Laboratories, Inc, CA, USA), as described in the manufacturer's instructions. Extracted DNA was purified with PowerClean DNA Clean-Up Kit (MIOBIO Laboratories, Inc, CA, USA), and then the DNA sample was diluted 10 times with distilled water.

2.4 Real-time PCR

The *amoA* genes of AOB and AOA were amplified with previously described primers (Rotthauwe et al., 1997, Tourna et al., 2008). Copy numbers of 16S rRNA gene of ANAMMOX bacteria were quantified with the real-time PCR assay(Tsushima et al., 2007).

Reaction mixtures of 20 µl contained 10 µl of SYBR Premix Ex Taq II (Takara, Japan), 32 pmol of forward and reverse primers, and 5 µl of template. Amplification, detection, and data analysis were performed Chromo4 (Bio-Rad laboratories BV, The Netherlands). The amplification program used for AOA was as follows: 94˚C for 5 min; 50 cycles of 30s at 94˚C, 30 sec at 56˚C, and 1 min at 72˚C. That for AOB was as follws: 95˚C for 5 min; 50 cycles of 30s at 95˚C, 1 min at 55˚C, and 1 min at 72˚C. The amplification program used for ANAMMOX bacteria was as follows: 50˚C for 2 min, 94˚C for 10 min; 50 cycles of 15s at 94˚C, 1 min at 60˚C. The PCR cycle

after which the fluorescence signal of the amplified DNA is detected (threshold cycle[C_T]) was used to quantify the concentration of AOB and AOA *amoA* gene copies. Quantification was based on comparison of the sample C_T value with the C_T values of a calibration carve based on known copy numbers of the *amoA* gene of AOB or AOA. The AOB numbers were calculated by assuming two amoA gene copy numbers per cell (Chain et al., 2003) and the AOA numbers by assuming one amoA gene copy number per cell.(Mincer et al., 2007)

2.5 Phylogenetic analysis

Phylogenetic analysis of the amoA genes of AOA were performed on soil sampled at the third step in condition C. DNA was isolated from the soil samples, and the *amoA* gene of AOA were amplified as described above. DNA products were purified using the DNA clean-up kit (PowerClean, MO BIO) according to the supplier's protocol. ExoSAP-IT (Amersham Biosciences, Tokyo, Japan) was used to remove the excess primers and dNTPs, and the ABI PRISM 3130 ×l Genetic Analyzer (Applied Biosystems) was used for sequencing. Sequence accuracy was confirmed by 2-directional sequencing. Phylogenetic analysis was performed with MEGA version 3.1 using the neighbor-joining method following the alignment of the archaeal *amoA* sequences using Clustal W.

3. Results and Discussion

3.1 Ammonia oxidizing microbes in the CW

Fig. 3 shows the results of number of ammonium oxidizing microbes. Results of 3 types of wetlands were shown. These results showed that there were ammonia oxidizing microbes in all wetland. Ammonia oxidizing bacteria was dominant. AOB in the wetland C was more than the others. There were 10^2-10^3 cells/cm^3. Additionally, AOA was detected in the wetland C. Leininge et al. (2006) reported that AOA might be the most abundant ammonia-oxidizing organisms in soil ecosystems on Earth. Park et al. (2006) reported that AOA was detected from the wastewater treatment plants. The constructed wetland was consisted of sand, and wastewater was inflow. These conditions were enable to AOA could grow in the CW. In this result, AOA was detected only from the wetland C. The reason why was not obvious. However, water level of the wetland C was higher than the other. It is possibility that the difference of water level was affected on the amount of ammonia oxidizing microbes.

Number of ANAMMOX bacteria was around quantification limits. Number of ANAMMOX bacteria of B type constructed wetlands in October was more than 10^2 cells/cm^3. The optimal temperature of ANAMMOX growth is known from 20 to 43 degree C (Strous, 1997). In our results, the temperature in December was 6 degree C. That is why, the number of ANAMMOX in December were very low. Fig. 4 shows TN removal from October to December. TN removal in October is higher than that in December. Dong et al.(2007) reported that the depth of 80 cm conventional vertical flow beds into a 25 cm un-

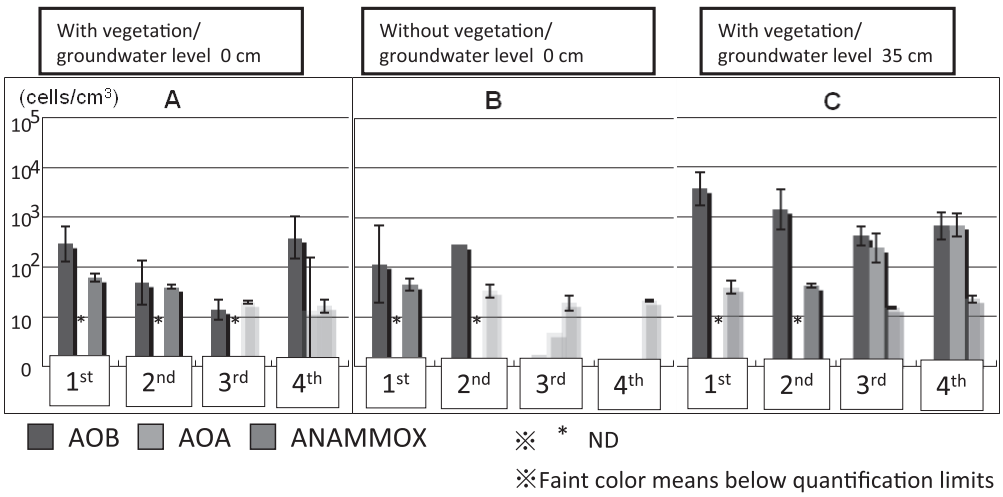

Fig. 3. Number of ammonia oxidizing microbes in December. Blue bars show the number of ammonia oxidizing bacteria. And orange bars show ammonia oxidizing archea.
Green bars show anammox bacteria.

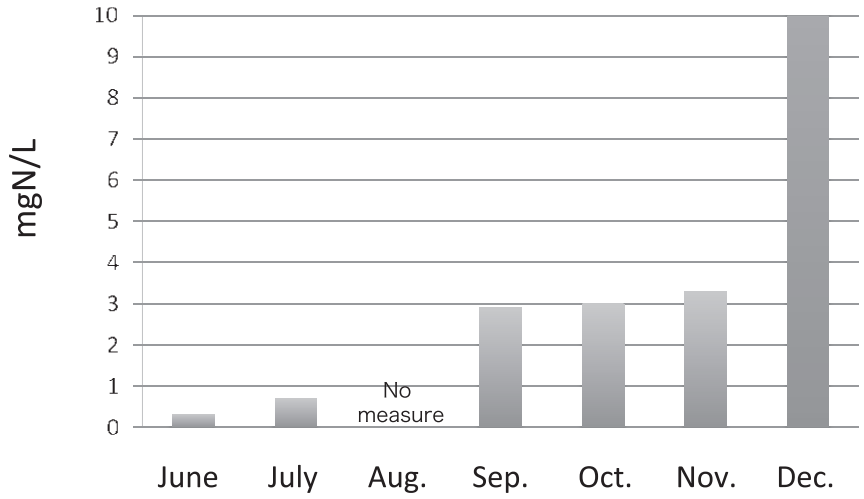

Fig. 4. TN concentrations of treated water thorough the 5th step of the CWs.

saturated layer and a 55 cm saturated layer, this new model cannot only get betted performance in removal efficiency of total nitrogen, but also achieved a promoting growth of ANAMMOX bacteria which will function on further removal the ammonium in horizontal flow bed. This report showed that the amount of ANAMMOX was related the removal of nitrogen. Comparing the TN removal and number of ANAMMOX, higher TN removal was observed when number of ANAMMOX is higher. This result suggested that ANAMMOX bacteria contributed to TN removal in the constructed wetlands.

3.2 Removal of coliform bacteria using CW

Fig.5 shows the number of coliform bacteria. After the start of operation, the coliform bacteria count at each stage 1-2log units decreased by about the fifth in the treated water is less 10 CFU/ml, showed high removal efficiency of coliform bacteria. After the rotation operation, a decrease in the number of coliform in each stage was about 0-1log. The numbers of coliform bacteria in treated water were from 10^3-10^4 CFU/ml. Coliform bacteria could be detected in soils of the constructed wetlands with average count 10^5 CFU/g. This may suggest that coliform bacteria

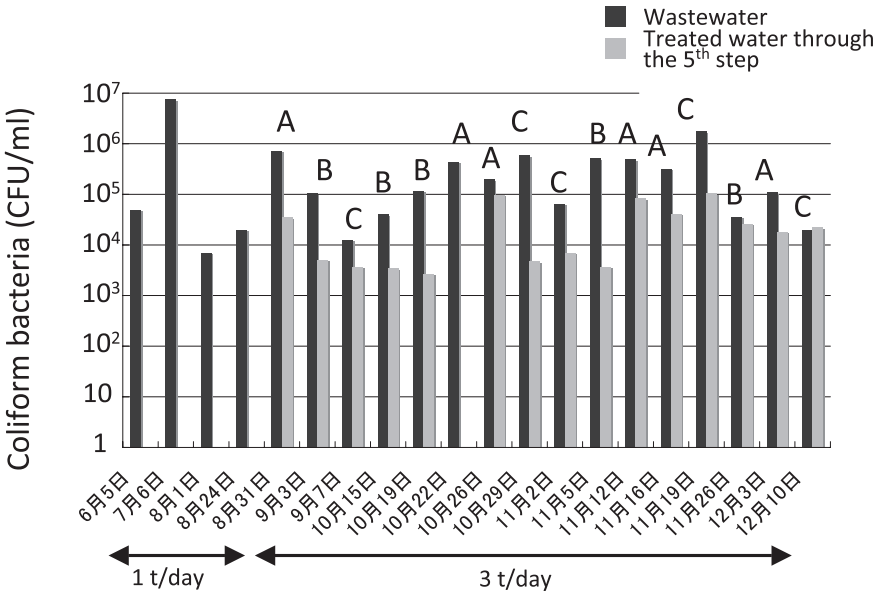

Fig. 5. Seasonal changes of number of coliform bacteria. A, B, C means type of constructed wetlands. From September, rotation running was started, so wastewater inflow increased up to 3 t/day. black bar show the number of coliform in wastewater, and gray bar show the number of colifrom in treated water through the 5th steps.

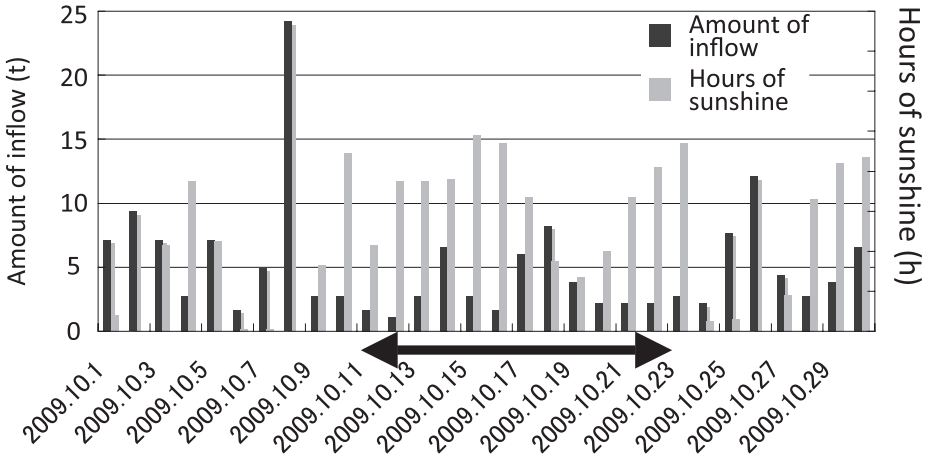

Fig. 6. Hours of sunshine and loading amount of wastewater and rain. Black bar show amount of inflow. Gray bar show hours of sunshine.

derived from the soil flow out when wastewater go through the wetlands. However only on October 22, showed a good reduction. Removal of coliform bacteria is 1-2 log units for one-stage systems and increases to 5.5 log units for multi-stage systems. From the analysis of the relationship between factors such as water quality and sunshine duration etc, coliform count, tended to decrease the number of coliform bacteria at times of low water inflow and longer daylight hours (Fig. 6). Thus, suggesting that contribute to reduction in the number of coliform bacteria in soil drying. Reed (*Phragmites* sp.) was known that high rate of transpiration (Larcher, 2004). Comparing between reed and surface of river, transpiration of reed was more than 10 times higher than quantity of evaporation from surface of river (Oshibe, 2004). Vegetation at the constructed wetland contributed to dry the soil in the wetland.

3.3 Recycle of wastewater

The agricultural water standard for rice cropping in Japan requires that TN concentation was below 1 mg/L. TN concentrations by the constructed wetland Kawatabi, were lower than 1 mg/L from June to August. When loading rate was 1 t/day, and it was warm seasons, treated water by the CW can meet the regulations for agricultural water.

The reclaimed to use for landscape water, the number of coliform bacteria have to lower than 1000 CFU/100 ml. When loading rate was 1 t/day, the number of coliform bacteria was lower than 100 CFU/100 ml. When loading amount was about 1 t/day and summer time, treated water can use for reclaimed water as landscapes water.

Conclusions

To reuse animal wastewater, nitrogen and coliform bacteria have to be removed. In this study, the number of ammonia oxidizing microbes and coliform bacteria in the constructed wetlands were investigated. Ammonia oxidizing bacteria was dominated in ammonia oxidizing microbes of the constructed wetland. ANAMMOX bacteria was detected in October from the constructed wetland, it is considered that ANAMMOX bacteria contributed to remove nitrogen in the constructed wetland. It is considered that soil-drying was one of the important factors for removing coliform bacteria. Treated water with the constructed wetland Kawatabi can use for reclaimed water as landscapes water, when the loading rate of wastewater was 1 t/day in warm season.

Reference

Chain, P., Lamerdin, J., F. Lamrimer, W. Regala, V. Lao, M. Land, L. Hauser, A.Hooper, M. Klotz, J. Norton, L. Sayavedra-Soto, D. Arciero, N. Hommes, M. Whittaker and D. Ar. (2003) Complete genome sequence of the ammonia-oxidizing bacterium and obligate chemolithoautotroph Nitrosomonas europaea. J. Bacteriol., 185: 2759-2773.

Dong, Z. and Sun, T. (2007) A potentioal new process for improving nitrogen removal in constructed wetlands- Promoting coexistence of Partial- nitrification and ANAMMOX. Ecological Engineering,

31: 69-78.

FAOSTAT Food Supply; http://faostat.fao.org/site/345/default.aspx

Hammer, D. A. (1993)Constructed wetlands for wastewater treatment: an overview of a low cost technology. Presented at the Constructed Wetlands for wastewater treatment conference, Middleton, Country Cork, Ireland.

Larcher, W. (2004) Plant physiological ecology, ver.2, Springer

Leininger, S., T. Urich, M. Schloter, L. Shwark, J. Qi, G. W. Nicol, J. L. Prosser, S. C. Schuster and C. Schleper (2006) Archaea predominate among ammonia-oxidizing prolaryotes in soils. Nature, 442(17): 806-809.

Mincer, T. J., M. J. Church, L. T. Taylor, C. Preston, D. M. Karl and E. F. DeLong (2007) Quantitative distribution of presumptive archearl and bacterial nitrifiers in Monterey Bay and the North Pacific Subtropical Gyre. Environ. Microbiol. 9: 1162-1175.

NRCS (1991) Technical requirements for agricultural wastewater treatment. Natl. Bull., No.210, 1-17, Natural Resource Conservation Service, United States Department of Agriculture, Washington, DC.

NRCS (1992) Agricultural waste management field handbook, Part 651. National Engineering Handbool 210-. Natural Resource Conservation Service, United States Department of Agriculture, Washington, DC.

Oshibe, R. and K. Kan (2004) Effect of reed field on reduction of streamflow. Japan Society Civil Engineers, vol.59, Disk1, 523-524.

Park, H., G. F. Wells, H. Bae, C. S. Criddle and C. A. Francis (2006) Occurrence of Ammonia-Oxidizing Archaea in Watewater Treatment Plant Bioreactors. Applied Enviorn. Microbiol., 72(8): 5643-5647.

Rotthauwe, J., K. Witzel and W. Liesack (1997) The ammonia monooxygenase structural gene amoA as a functional marker: molecular fine-scale analysis of natural ammonia-oxidizing populations. Appl. Environ. Microbiol., 63: 4704-4712.

Strous, M., E. V. Gerven, P. Zheng, J. G. Kuenen and M. S. M. Jetten (1997) Ammonium Removal from Conecentrated Waste Streams with the Anaerobic Ammonium Oxidation (ANAMMOX) Process in Different Reactor Configuration. Water Research, 31(8): 1955-1962.

Tourna, M., T. E. Freitag, G. W. Nicol and J. L. Prosser (2008) Growth, activity and temperature responses of ammonia-oxidizing archaea and bacteria in soil microcosms. Environmental Microbiology, 10:1357-1364.

Tsushima, I., T, Kindaichi and S. Okabe (2007) Quantification of anaerobic ammonium-oxidizing bacteria in enrichment cultures by real-time PCR. Water Research, vol. 41:785-794.

Effect of Grazing on Habitat and Behavior of Wild Mammals

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Abstract

Although there are few grassland specialists among terrestrial mammal species (110-115) in Japan, 52 mammal species inhabit Japanese grasslands and 22 of them are listed in the Red Data Book of Japan. However, some grassland mammals, such as the sika deer and the wild boar, cause serious damage to grasslands. The relationship between cattle grazing and wild mammals in Japanese grasslands should be considered within the following two contexts: 1) the conservation of grassland mammals and 2) the control of mammal damage in grasslands. Firstly, the effect of cattle grazing on the habitats of small mammals was considered in the context of the conservation of grassland mammals. The habitat quality for small mammals was high in tall-grass pasture with low grazing intensity but was low in short-grass pasture with high grazing intensity. Therefore, there is a trade-off between the habitat quality for small mammals and the productivity for grazing cattle in the sympatric animal production system. Secondly, the zoning of areas for cattle grazing between wildlife habitats and agricultural areas was considered in order to control wildlife damage in grasslands. In abandoned cultivated land, the introduction of cattle grazing was an effective deterrent to rooting by wild boars, because cattle grazing reduced the food resources and the secure cover for wild boars. However, the intrusion of sika deer into pastures could not be prevented by cattle grazing. The activity of sika deer within a stock farm was affected by the herbage quality and the nearest escape cover. Hence, cattle grazing alone is not sufficient for preventing wildlife damage in grasslands.

Japanese wild mammals in grassland

Japan is a mountainous and forest-dominant country. Mountainous and forested areas cover 61 and 66% of the land, respectively. Grassland covers only 3% of the land area of Japan. Furthermore, Japanese grassland is not a native but a semi-natural grassland maintained by various human activities such as cattle grazing, harvesting and burning (Tsukada et al. 2004a). Therefore, the Japanese mammal fauna reflect these habitat characteristics. Although a total of 52 wild mammal species are listed as inhabitants of grassland in Japan (Tsukada 2007), making up about 42.3% of all terrestrial mammal species (115 species; Abe et al. 2005), only two species, viz. harvest mouse (*Micromys minutus*) and Japanese field vole (*Microtus montebelli*), are grassland specialists and the other species are habitat generalists (Tsukada 2007). Among these grassland mammal species, 22 species (42.3%) are listed in the Japanese Red List (Ministry of the Environment, Japan 2007), and 33 species (63.5%) are ranked as “common” in the Red List of the Mammalogical Society of Japan (Mammalogical Society of Japan 1997). Two grassland specialist species, the harvest mouse and Japanese field vole, are not listed in the Japanese Red List but are listed in 27 and 9 local Red Data Books, respectively. The semi-natural grasslands, which are important habitats for such grassland mammal species, are not stable and have been diminished because the agricultural activities in the grassland have also been abandoned in the course of recent rapid socio-economic changes.

On the other hand, other grassland mammal species have become abundant and caused some serious problems. Among 13 of the medium and large-sized grassland species observed in pastures in Tochigi Prefecture, central Japan (Tsukada et al. 2008a), sika

deer (*Cervus nippon*) and wild boars (*Sus scrofa*) have caused especially serious damage in grasslands. Sika deer graze on herbage stocked for cattle with an economic cost amounting to ¥22.3 billion, which represents 86.8% of the total herbage damage caused by wild mammals in 2008 (MAFF 2009). For example, the amount of herbage damage caused by sika deer was estimated at about 245-359 tons of dry matter within a year at the local farm, Koze Dairy Farm, which encompasses 99.4 ha of grassland (Table 1). In contrast, wild boars have caused damage chiefly by their rooting activity in grasslands, although they also graze on herbage for cattle (Ueda et al. 2008). The wild boar is omnivorous and prefers eating the roots of plants, such as clover and dandelion, and also eats the beetle larvae of Rutelinae. When wild boars forage on these plants and insects, they turn up grass roots (Ide and Nakagami 2009a, b; Fig. 1).

Given the current situation regarding grassland mammals described above, the relationship between cattle grazing and wild mammals in Japanese grasslands should be considered within the following two contexts: 1) the conservation of grassland mammals and 2) the control of mammal damage in grasslands. With regard to the first context, I will discuss how to manage cattle grazing in accord with the conservation of mammals in grasslands. With regard to the second context, I will examine cattle grazing as a possible management tool for controlling damage by wild mammals.

The conservation of small grassland mammals

Firstly, the effect of cattle grazing on the habitats of small mammals was considered in the context of the conservation of grassland mammals. Tsukada et al. (2004b) investigated the species composition of the small mammal fauna in pastures and its relationship with pasture characteristics in the northern part of Tochigi, Japan. In this study, the species richness of small mammals was high in tall-grass pastures while it was low under high cattle grazing intensity (Fig. 2). This relationship was also observed with respect to the micro habitat features within pastures. The number of large Japanese field mice (*Apodemus speciosus*) increased with an increase in the sward height where the animal was captured. These results suggest that the habitat quality for small mammals is not fully compatible with cattle grazing. When the maximum

Table 1. Herbage damage by sika deer at Kouzu Dairy Farm in Gunma Prefecture, Japan[1].

Year	2007	2008	2009
Amount of herbage damage (DMt)	285	245	359
Cost for damage (¥ Mill.)[2]	13.3	11.5	16.8

[1]Herbage damage was estimated by the difference in herbage yield between caged and uncaged plots (Tsukada and Ishikawa in preparation)

[2]The cost was converted at the herbage purchase price (¥46.7/kg)

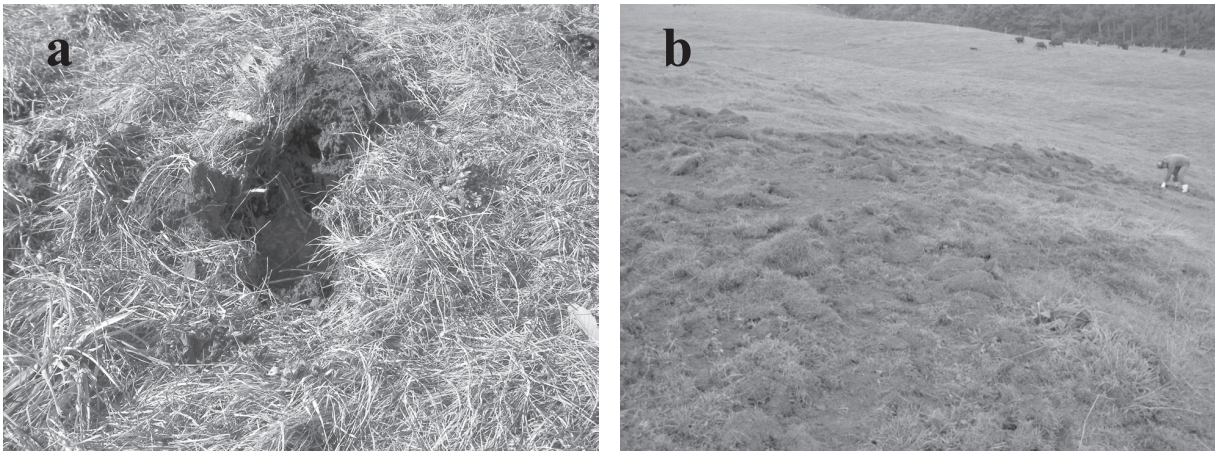
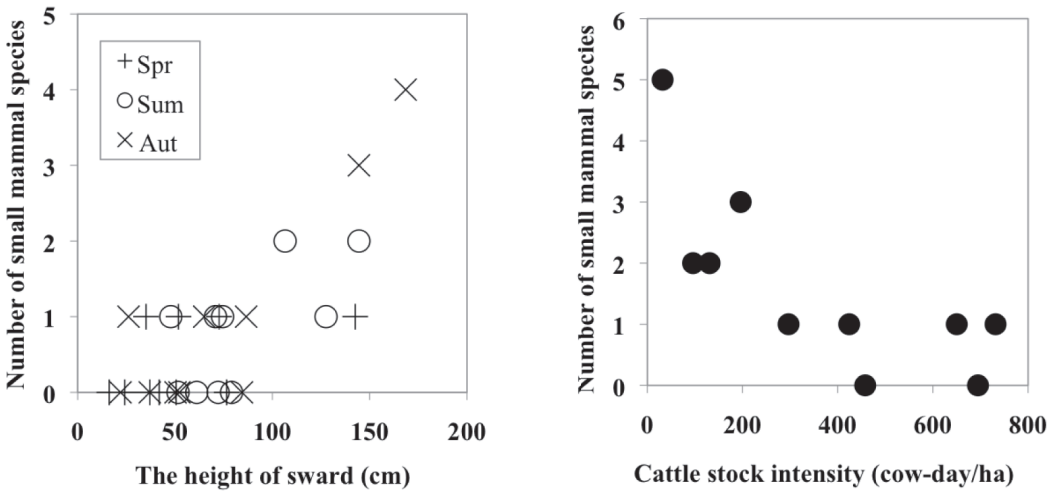


Fig. 1. Two types of rooting activity by wild boars in grasslands. The perforation (a) and exfoliation (b) types of digging.



Revision from Tsukada et al. (2004b)

Fig. 2. Relationship between species richness of small mammals and two pasture characteristics: 1) maximum plant height (left) and 2) cattle stock intensity (right).

productivity for cattle grazing is expected, the grazing intensity would become high and the short-grass pasture would be maintained. However, in such situations, the habitat quality for small mammals becomes worse because of the high predation risk. On the other hand, when high habitat quality for small mammals is expected, tall-grass pasture with low grazing intensity becomes dominant, and the productivity for grazing cattle becomes worse. In other words, there is a trade-off between the habitat quality for small mammals and the productivity for grazing cattle in the sympatric animal production system. Therefore, we must carefully consider the trade-offs when the conservation of grassland mammals is considered in a pasture where animal production is conducted concurrently.

Mammal damage control in grassland

The control of mammal damage to grassland must also be considered. Although many counter measures for controlling mammal damage have been proposed and actually put into practice in grasslands (Tsukada 2009), the application of cattle grazing as a possible management tool for controlling damages inflicted by wild mammals has increased recently in Japan (Senda et al. 2002; Ueda 2003; Arita 2005). The basic concept of this use of cattle grazing is shown in Fig. 3. In this schema, wildlife habitat is mostly located in the mountainous area where pest mammals, such as wild boars, sika deer and Japanese monkeys, are abundant.

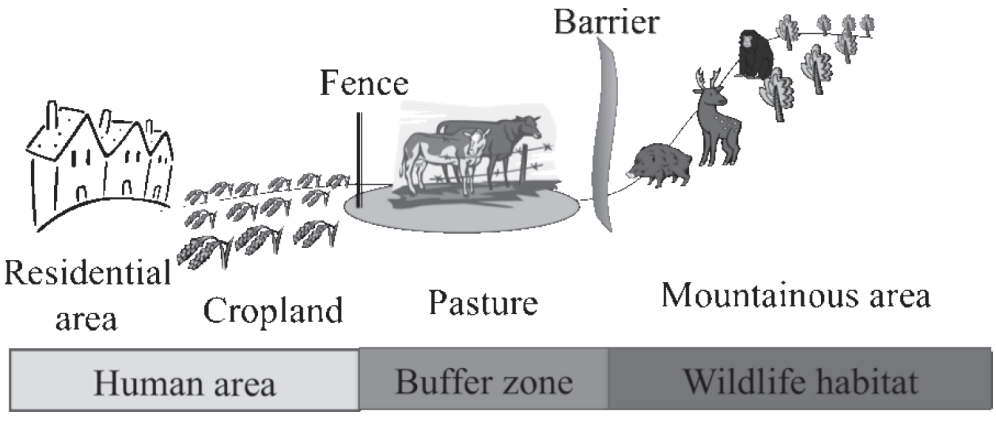
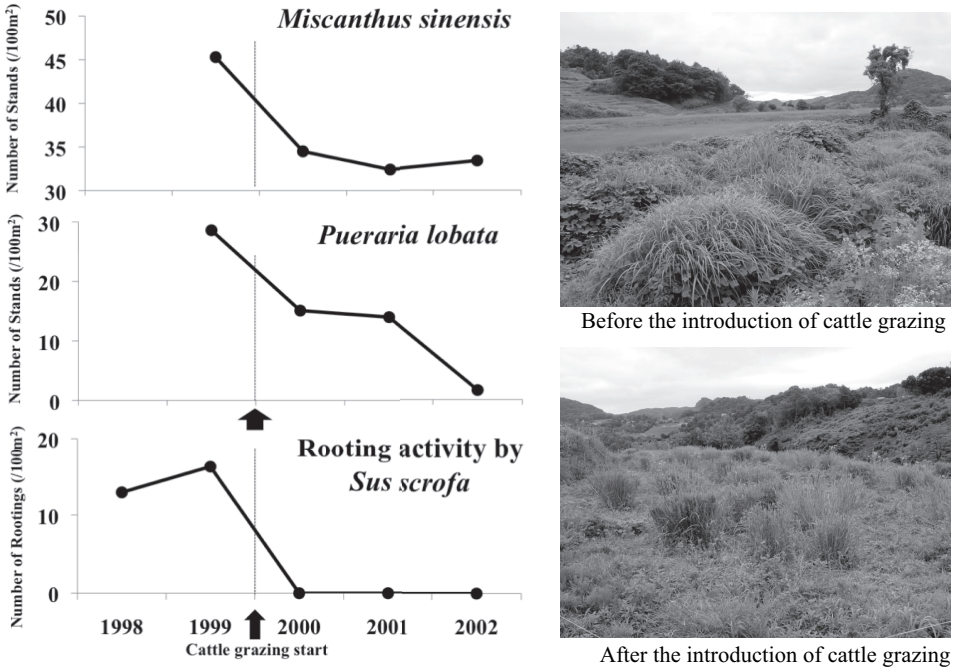


Fig. 3. A schema of the control of wildlife damage by cattle grazing in pasture.

The human areas, including croplands and residential areas, are located adjacent to the mountainous area. The pastures are located just between the human areas and the wildlife habitats. The pasture can be a buffer zone which can prevent pest mammals from intruding into the human area. In fact, in some abandoned cultivated land, the introduction of cattle grazing effectively prevented the infliction of damage by wild boars (Ide 2007). Ide et al. (2005) investigated the rooting activities of wild boars and the main flora in abandoned cultivated land located in the middle of Shimane Prefecture in southwestern Japan before and after the introduction of cattle grazing. Before the introduction of cattle grazing, the abandoned cultivated land was dominantly covered by Japanese plume-grass (*Miscanthus sinensis*) and kudzu-vine (*Pueraria labata*), and the amount of rooting by wild boars was relatively high (Fig. 4). However, after cattle grazing was introduced in this location in 2000, the Japanese plume-grass and kuzu-vine vanished, and the rooting activity by wild boars decreased dramatically (Fig. 4). These results showed that cattle grazing in abandoned cultivated land had an effect on the habitat and behavior of wild boars. The habitat changes had two aspects. One was that the food resources for wild boars, such as the root of the kudzu-vine, had decreased. The second change was that the secure places for wild boars, such as bushy Japanese plume-grass, had also disappeared. Because of these habitat changes, the rooting activity of wild boars also decreased after the grazing by cattle was introduced.

The effect of cattle grazing in controlling herbage damage induced by sika deer has been the subject of several studies. Tsukada et al. (2008b) investigated the relationship between the appearance of sika deer and cattle grazing in the pastures on two farms located in Tochigi Prefecture, Japan. The deer activities as estimated by camera trapping did not appear to be affected by the cattle grazing intensities on the pastures of both farms. The captivity study also showed that sika deer first showed various avoidance behaviors in relation to cattle, but became habituated to the cattle after two weeks of exposure to them (Matsumot et al. 2005). In other cases, the appearance of sika deer in a pasture accompanied by grazing cattle was observed in various regions, while the same situation was also observed for wild boars (Fig. 5). On the other hand, deer activity within a boundaries of a farm was clearly affected by pasture utilization, such as pasture renovation. Tsukada et al. (2009) investigated the



Revision from Ide et al. (2005)

Fig. 4. The change in the rooting activity by wild boars and the main flora on abandoned cultivated land located in the middle of Shimane Prefecture in southwestern Japan before (above photo) and after (below photo) the introduction of cattle grazing. Dr. Ide took these photos.

Fig. 5. The appearance of wild sika deer (left) and wild boars (right) at pastures accompanied by grazing cattle. The left photo was taken at the public pasture located at Izu, Shizuoka, central Japan. The right photo was taken by Ishimoto (National Livestock and Breeding Center) at National Agricultural Research Center for Western Region located at Shimane, western Japan.

activity of sika deer within a farm. They compared the density of deer feces among locations at various distances from the farm periphery. As a result, an extraordinarily high density of deer feces was observed in only one paddock where pasture renovation had been conducted, although the distance from the farm periphery did not affect the density of deer feces. Pasture renovation generally improves the quantity and quality of herbage in a pasture (Hopkins et al. 1990; Lee et al. 2000). Therefore, deer are assumed to forage selectively in renovated pastures. The activity of deer in open areas was also reported to be affected by the distance from secure places (Reynolds 1966; Williamson and Hirth 1985; Takatsuki 1989).

As is described above, although allowing cattle to graze alone is not sufficient to prevent damage by sika deer nor wild boars in grasslands, the behaviors of these wild mammals could be affected and modified through changes in their habitats and food resources due to the effects of cattle grazing.

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References

Abe, H., N. Ishii, T. Itoo, Y. Kaneko, K. Maeda, S. Miura and M. Yoneda (2005) A Guide to the Mammals of Japan. Tokai University Press, Kanagawa, 206.

Arita, H. (2005) Abandoned paddy field management: functions of cattle grazing and land use. Trans. JSIDRE, 253: 51-58 (in Japanese with English summary).

Hopkins, A., J. Gilbry, C. Dibb, P. J. Bowling and P. J. Murray (1990) Response of permanent and reseeded grassland to fertilizer nitrogen. 1. Herbage production and herbage quality. Grass Forage Sci., 45: 43-45.

Ide, Y. (2007) Effect of cattle grazing on the rooting by wild boars. Jpn. J. Grass. Sci., 53: 59-63 (in Japanese).

Ide, Y., N. Koyama, Y. Takahashi and H. Kobayashi (2005) Effect of cattle grazing on the rooting by wild boars in abandoned cultivated land. Bull. WeNARC, 4: 173-181 (in Japanese with English summary).

Ide, Y. and K. Nakagami (2009a) Rooting by wild boars in temperate meadow 1. Seasonal occurrence of perforation type rooting and cause of such a digging. Jpn. J. Grass. Sci., 55 (Suppl): 101 (in Japanese).

Ide, Y. and K. Nakagami (2009b) Rooting by wild boars in temperate meadow 2. Seasonal occurrence of exfoliation type rooting and cause of such a digging. Jpn. J. Grass. Sci., 55 (Suppl): 102 (in Japanese).

Lee, I. D., H. S. Lee and S. K. Kim (2000) Comparative studies on the DM yield and quality before and after pasture renovation of summer depression damage pasture. J. Korean Soc. Grass Forage Sci., 20: 215-220.

MAFF (Ministy of Agriculture, Forestry and Fisheries) (2009) The 2008 Situation of Crop Damages by Wildlife. MAFF, Tokyo, Japan. http://www.maff.go.jp.proxy.blastsound.com/j/seisan/tyozyu/higai/h_zyokyo/h20/pdf/100212-a.pdf (date of ac-

cess: October 18, 2010) (in Japanese)

Mammalogical Society of Japan (1997) Red Data of Japanese Mammals. T. Kawamichi (Ed.) Bun-ichi Sogo Shuppan, Tokyo, 279 (in Japanese with English list).

Matsumoto, A, K. Takeda, S. Hayama and K. Ohshima (2005) Avoidance behavior of captive sika deer against the presence of cattle. Jpn. J. Grass. Sci., 50 (Suppl): 400-401 (in Japanese).

Ministry of the Environment, Japan (2007) Red List of Mammal. Ministry of the Environment, Japan, Tokyo. http://www.env.go.jp/press/file_view.php?serial=9941&hou_id=8648 (date of access: October 26, 2010) (in Japanese).

Reynolds, H. G. (1966) Use of ponderosa pine forest in Arisona by deer, elk and cattle. USDA, Forest Service, Rocky Mountain Forest and Range Experiment Station, Fort Collins, Colorado. Research Note RM-63: 1-7.

Senda, M., Y. Tanimoto and N. Koyama (2002) Grazing system for conservation of rural resources -On the analysis of case studies-. Misc. Pub. Natl. Agric. Res. Cent., 1: 1-74 (in Japanese with English summary).

Takatsuki S. (1989) Edge effects created by clear-cutting on habitat use by sika deer on Mt. Goyo, northern Honshu, Japan. Ecol. Res., 4: 287-295.

Tsukada, H. (2007) The feature, current status and conservation issues of wild mammals in Japanese grassland. Jpn. J. Grass. Sci., 53: 52-58 (in Japanese).

Tsukada, H. (2009) Conservation of wild mammals and the control of wildlife damage. The standards for grassland management -Multifunctionality of grassland. Japan Grassland Agriculture and forage Association, Tokyo, 151-161 (in Japanese).

Tsukada, H., O. Imura and K. Shi (2004a) Conservation and management of grassland biodiversity in East Asia. S. H. Hong, J. A. Lee, B.S. Ihm, A. Farina, Y. Son, E. S. Kim and J. C. Choe (Eds.) Ecological Issues in a Changing World: Status, Response and Strategy. Kluwer Academic Publishers, Netherland, 157-172.

Tsukada, H., T. Imura, M, Sutoh, T. Kosako and M. Fukasawa (2004b) Small mammal fauna of public pastures in northern Tochigi, Japan. Grass. Sci., 50: 329-335.

Tsukada, H., M. Fukasawa, T. Kosako, M. Sutoh and T. Imura (2008a) Mammal fauna survey by camera trapping at pastures in Tochigi prefecture, Japan. M. Nashiki, S. Itano, S. Sakanoue, M. Tsutsumi, K. Shimoda and S. Kano (Eds.), Report on the Grassland Dynamics in Japan (The 7th Report), National Institute of Livestock and Grassland Science, Nasushiobara, 110-115.

Tsukada, H., M. Fukasawa and T. Kosako (2008b) Is cattle grazing an effective deterrent against sika deer (*Cervus nippon*) intrusion into pastures? Grass. Sci., 54: 45-51.

Tsukada, H., M. Fukasawa, T. Kosako, Y. Nakamura and Y. Hanafusa (2009) Effect of distance from farm periphery on the risk of forage damage by sika deer (*Cervus nippon*). Grass. Sci., 55: 193-199.

Ueda, E. (2003) Countermeasure for wildlife damage prevention using zoning with cattle grazing. Takahashi, S. (Ed.) Wild Mammals in Shiga -Coexistence with Human. Sunrize-Syuppan, Shiga, 132-157 (in Japanese).

Ueda, H., Y. Takahashi and M. Inoue (2008) Temperate grassland provides winter food for wild boar (*Sus scrofa* L.). Jpn. J. Grass. Sci., 54: 244-248 (in Japanese with English summary).

Williamson, S. J. and D. H. Hirth (1985) An evaluation of edge use by white-tailed deer. Wildl. Soc. Bull., 13: 252-257.

Archaeal Community during Cattle Manure Composting Process in Field-scale Facility

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Abstract

Composting process is a useful technique to transform cattle manure into organic fertilizer. During the process, complex microbial communities consisting of bacteria, archaea, and fungi carried out biodegradation of substrates. Because it has been considered that bacteria play an important role in composting, their community structure was studied well. However, archaeal one has not been identified clearly. To understand their community structure and abundance, cattle manure was composted in field-scale facility and composting materials were analyzed by culture-independent approaches.

Clone library constructed from archaeal 16S rRNA genes showed that archaeal community in compost was mainly consisted of methane-producing archaea (methanogen) and ammonia-oxidizing archaea (AOA). During first 2 days, clones which were related to methanogens in the animal rumen or manure were detected, suggesting that fecal methanogen could survive in the early stage of composting. Other methanogen, which grouped into thermophilic *Methanosarcina* spp. were present thoroughout the process, indicated that they might adapt the environmental changes such as high temperature. AOA-like sequences were detected from all investigated samples. They showed high identity with cultured AOA originated from hot spring. In this study, we revealed the changes in archael community in the composting process. It was also suggested that AOA could actively involve in nitrification of composting systems.

Introduction

Livestock manure accounts for a large part of the total waste generated in the livestock industry (Haga, 1999). Animal manure should be treated properly because huge amount of manure can cause environmental problems such as air, water, and soil pollution (Bernal et al., 2008). Composting of animal manure is one of the most effective techniques in terms of mineralization of organic components, microbial stabilization, and removal of odors and so on (Bernal et al., 2008). In addition, final product can apply to agricultural soil as high quality fertilizer (Bernal et al., 2008). Composting is a biological process involving various microorganisms (Insam and de Bertoldi, 2007). To follow microbial community whose structure and diversity change dramatically during composting, researchers recently use culture-independent techniques like DNA analysis. It can detect both uncultured and cultured organisms from compost while culture dependent one can only detect about 8.5% of total microbes (Gong et al., 2005). By using this approach, the changes in the bacterial community structure during the composting of animal manure were analyzed well (Guo et al., 2007; Yamada et al., 2008; Yamamoto et al., 2009) because it has been considered that bacteria play an important role with various characteristics. On the other hand, archaea had been recognized as minor components of the microbial community in compost because they mainly live in thermophilic or anaerobic environments (Insam and de Bertoldi, 2007). But some reports showed considerable methane production from cattle manure compost (Hao et al., 2001). Others indicated the pres-

ence of methane-producing archaea (methanogen) from composting of organic waste (Thummes et al., 2007) or rice straw (Cahyani et al., 2004). Moreover, new archaeal species called ammonia-oxidizing archaea (AOA) was discovered living in moderate environments like seawater and soil and shown as essential actor in nitrogen cycle in various environments (Prosser and Nicol, 2008). Therefore, archaea can be also considered as an essential component of the microbial community in compost. Indeed, we detected archaeal *amoA* gene encoding ammonia monooxygenase subunit A from cattle manure compost (Yamamoto et al., 2010). However, there is no study about whole archaeal community in composting of animal manure.

In this study, we analyzed the archaeal community structure during composting process of cattle manure using culture-independent techniques to evaluate its composition and how it changed.

Materials and methods

Composting process and sampling

The composting experiment was performed at a field-scale facility in Field Science Center, Graduate School of Agricultural Science, Tohoku University (Miyagi, Japan). For composting experiment, 1,140 kg of dairy cattle manure and 230 kg of sawdust were used. The mixture was then piled and stirred with a shovel loader 3 or 5 times per week for 30 days.

Analysis of chemical and physical parameters

The temperature was automatically measured with a temperature/humidity data logger (TR-71S; T&D Corporation, Nagano, Japan) at a depth of 30 cm from the surface. Moisture content was determined by measuring weight of samples after these were placed in drying oven at 105˚C overnight. PH was measured using a pH meter (WM-22EP; DKK-TOA Corporation, Tokyo, Japan).

DNA extraction

We selected 6 samples (day 0, 2, 6, 16, 24, and 30) for DNA extraction. Compost samples were collected at a depth of 30 cm from the surface before stirring and transferred to the laboratory on ice. All samples were freeze-dried overnight using a freeze dryer (FDU-830; Tokyo Rikakikai Co. Ltd., Tokyo, Japan) to maintain the water content at a low level and prevent loss of DNA during storage (Miller et al., 1999; Reuter et al., 2009). Total DNA was extracted from 0.025 g freeze-dried compost using PowerSoilê DNA Isolation kit (MO Bio Labs, Inc., Carlsbad, CA, USA). The extracted DNA was then dissolved in TE buffer (10 mM Tris-HCl, 1 mM EDTA).

PCR, cloning analysis, and sequencing

Approximately 1,400 bp of archaeal 16S rRNA gene was amplified using a primer set listed in Table 1. PCR was performed using Ex Taq (Takara Bio Inc., Shiga, Japan) with an iCycler (Bio-Rad Labs Inc., Hercules, CA, USA). PCR products with correct DNA fragments were cloned using Novagen Perfectly Blunt Cloning kits (EMD Chemicals Inc., San Diego CA, USA), according to the manufacturer's instructions. Clones with objective DNA fragments were then selected and amplified using the primer set that targets the cloned plasmid (pT7Blue vector, EMD Chemicals Inc.) (Table 1). PCR products were purified with ExoSAP-IT (USB Corporation, Cleveland, OH, USA). The purified products were sequenced using the BigDye Terminator Cycle Sequencing kit v.1.1 (Applied Biosystems, Foster City, CA, USA). Obtained products were analyzed using an ABI PRISM 3100-Avant Autosequencer (Applied Biosystems).

Table 1. Primer sets used in this study

Name	Sequence (5'-3')	Targets	Reference
Arch21f	TTCCGGTTGATCCY[1]GCCGGA	Archaeal 16S rRNA gene	DeLong (1992)
1492r	ACGGY[1]TACCTTGTTACGACTT	16S rRNA gene	DeLong (1992)
T7	TAATACGACTCACTATAGGG	Plasmid DNA	EMD Chemicals Inc. (WI, USA)
U19	GTTTTCCCAGTCACGACGT	Plasmid DNA	EMD Chemicals Inc. (WI, USA)

[1]C or T

Construction of clone library

The sequences were assembled using Seqscape software (Applied Biosystems). After chimeric sequences were removed from the library using the Pintail program, the analyzed sequences were compared to sequences registered in the database of the DNA Databank of Japan (DDBJ) using the BLAST www system (http://blast.ddbj.nig.ac.jp/top-j.html). They were divided into operational taxonomic units (OTUs) for sequences with >97% homology to each other (McCaig et al., 1999).

Results

Changes in chemical and physical parameters during the composting process

Changes in temperature and moisture content are shown in Fig. 1. The temperature was about 14˚C at the beginning of the composting process (Fig. 1a). After the examination was started, it increased rapidly within 2 days. The highest temperature was recorded on day 5 (77.9˚C) and kept >60˚C for 18 days. From day 21, the temperature began to decrease and reached 29.3˚C at the end. The initial moisture content was about 67% (Fig. 1b). It continued to decline from day 8 and reached low value (about 30%). The pH was mildly alkaline (8.2–8.9) during the composting process except for on day 0.

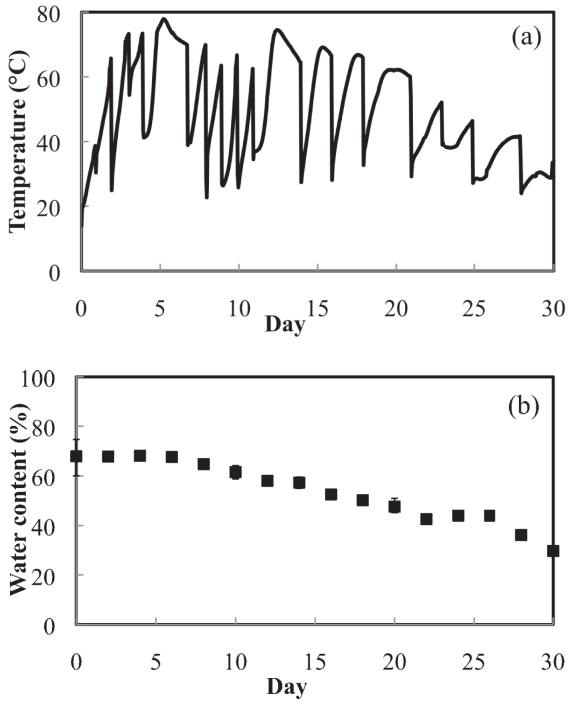

Fig. 1. Changes in (a) temperature and (b) water content during the composting process.

Archaeal community structure during the composting process

Number of sequenced clones was from 36 (day 6) to 78 (day 24). In total, 14 OTUs were generated and almost OTUs had most related species grouped into either the methanogens or AOA (Fig. 2). OTU1 was detected on day 0 and day 2 and nearly identical to the sequence originating from the groundwater. Other OTUs (i.e. OTU7) detected from only day 0 and day 2 were mainly related to uncultured methanogens originated from animal rumen or manure with relatively high homology. *Methanomicrococcus*-like sequences were obtained from only day 2 (OTU4). OTU2 was the large part of total clones during composting process. It was close to uncultured thermophilic *Methanosarcina* spp. with high homology while OTU1 was absent or observed at low abundance. OTU12 was detected from all investigated samples. It was closely related to '*Candidatus* Nitrososphaera gargensis' with high homology (98%). OTU13 and OTU14 had uncultured sequences as most relative sequences but closet sequences for both OTUs were obtained from same soil environment (Bintrim et al., 1997).

Discussion

Clone library indicated that both methanogen and AOA were the dominant archaeal species during the composting process of cattle manure. The archaeal community structure found in the present study had some differences from previous reports analyzing

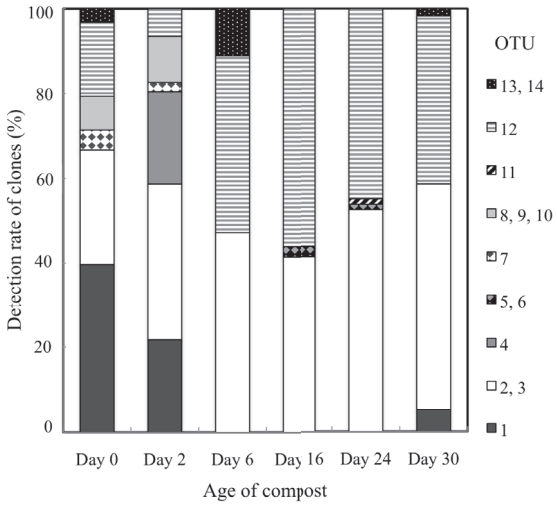

Fig. 2. Relative abundance of the OTUs in the composting materials. An OTU consisted of clones with more than 97% homology.

composting materials. For example, we detected some OTUs with sequences from animal waste and rumen as most relatives. Four OTUs (OTU4, 8–10) were closely related to uncultured methanogens found in animal rumen, intestine, and in anaerobic digesters (Snell-Castro et al., 2004).

OTU2 became the most dominant OTU in the clone libraries on days 6, 16, 24, and 30. These sequences related an uncultured clone from a thermophilic anaerobic waste digester (Tang et al., 2004) and grouped into themophilic *Methanosarcina* spp. Thummes et al. (2007) also detected some clones grouped into this cluster from different composting materials. Gattinger et al. (2007) reported themophilic *Methanosarcina*-like sequences were detected in fertilized soil. Thus, *M. thermophila* appears to adapt to the composting environment and increase its detection rate after other methanogens originated from cattle manure have decreased in abundance due to high temperature.

He et al. (2000) reported an anaerobic microsite inside composting food waste particles. Our results indicate that the methanogenic community was present within anaerobic sites of composting material under aerobic condition. In addition, methanogens might transit from compost to soil when compost is applied to soil (Cahyani et al. 2004; Gattinger et al. 2007).

It's notable that AOA-like sequences were detected throughout composting process. Thummes et al. (2007) analyzed about 120 clone sequences using composting materials, however, no sequence belonging AOA was detected. Our study indicated that AOA was an essential component of the archaeal community in compost, especially from days 6 to 30. OTU12 had high similarity to those of *Candidatus* Nitrososphaera gargensis, which obtained from hot springs (Hatzenpichler et al. 2008). One possible assumption is that the member of OTU12 might derive from finished composting materials produced in the same facility. It is highly possible that AOA usually present and is critical to nitrification in cattle manure compost since our previous study showed the existence of archaeal *amoA* gene encoding ammonia monooxygenase, the key enzyme responsible for ammonia oxidation (Yamamoto et al., 2010).

In the present study, archaeal community structure during composting process using cattle manure was displayed for the first time. Archaeal community was mainly consisted of methanogen and AOA. At the initial stage of composting, some methanogens originated from animal manure or rumen was dominated. After reaching high temperature, thermophilic *Methanosarcina*-like species were the most dominant methanogens because they could adapt to increasing temperatures. In addition, we detected AOA from compost for the first time and found that they existed throughout the composting process. It was also suggested that AOA could actively involve in nitrification of composting systems. This study provides the importance for studying archaeal community to understand microbiology of compost.

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References

Bernal, M. P., J. A. Alburquerque, and R. Moral (2009) Composting of animal manures and chemical criteria for compost maturity assessment. A review. Bioresour Technol, 100: 5444-5453.

Bintrim, S. B., T. J. Donohue, J. Handelsman, G. P. Roberts and R. M. Goodman (1997) Molecular phylogeny of Archaea from soil. Proc. Natl. Acad. Sci. USA, 94: 277-282.

Cahyani, V. R., K. Matsuya, S. Asakawa and M. Kimura (2004) Succession and phylogenetic profile of methanogenic archaeal communities during the composting process of rice straw estimated by PCR-DGGE. Soil. Sci. Plant. Nutr., 50: 555-563.

DeLong, E. F. (1992) Archaea in coastal marine environments. Proc. Natl. Acad. Sci.USA, 89: 5685-5689.

Gattinger, A., M. G. Höfle, M. Schloter, A. Embacher, F. Böhme, J. C. Munch and M. Labrenz (2007) Traditional cattle manure application determines abundance, diversity and activity of methanogenic Archaea in arable European soil. Environ. Microbiol., 9: 612-624.

Gong, C. H., J. Koshida, K. Inoue and T. Someya (2005) Fluorescence direct count of bacteria in various manures and composts as compared with plate count. Jpn J Soil Sci Plant Nutr, 76: 401-410.

Guo, Y., N. Zhu, S. Zhu, and C. Deng (2007) Molecular phylogenetic diversity of bacteria and its spa-

tial distribution in composts. J. Appl. Microbiol., 103:1344-1354.

Haga, K. (1999) Development of composting technology in animal waste treatment. Asian-Aus. J. Ani. Sci., 12: 604-606.

Hao, X., C. Chang, F. J. Larney and G. Travis (2001) Greenhouse gas emissions during cattle feedlot manure composting. J. Environ. Qual., 30: 376-386.

Hatzenpichler, R., E. Lebedeva, E. Spieck, K. Stoecker, A. Richter, H. Daims and M. Wagner (2008) A moderately thermophilic ammonia-oxidizing crenarchaeote from a hot spring. Proc. Natl. Acad. Sci. USA, 105: 2134-2139.

He, Y., Y. Inamori, M. Mizuochi, H. Kong, N. Iwami and T. Sun (2000) Measurements of N_2O and CH_4 from the aerated composting of food waste. Sci. Total Environ., 254: 65-74.

Insam, H. and M. de Bertoldi (2007) Microbiology of the composting process. L. Diaz, M. de Bertoldi, W. Bidlingmaier and E. Stentiford (Eds.), Compost Science and Technology, Elsevier Ltd., Oxford, UK, 26-48.

McCaig, A., L. Glover and J. Prosser (1999) Molecular analysis of bacterial community structure and diversity in unimproved and improved upland grass pastures. Appl. Environ. Microbiol., 65: 1721-1730.

Miller, D. N., J. E. Bryant, E. L. Madsen and W. C. Ghiorse (1999) Evaluation and optimization of DNA extraction and purification procedures for soil and sediment samples. Appl. Environ. Microbiol., 65: 4715-4724.

Prosser, J. I. and G. W. Nicol (2008) Relative contributions of archaea and bacteria to aerobic ammonia oxidation in the environment. Environ. Microbiol., 10: 2931-2941.

Reuter T., W. Xu, T. Alexander, K. Stanford, Y. Xu and T. McAllister (2009) Purification of polymerase chain reaction (PCR)-amplifiable DNA from compost piles containing bovine mortalities. Bioresour. Technol., 100: 3343-3349.

Snell-Castro, R., J. Godon, J. Delgenès and P. Dabert (2005) Characterisation of the microbial diversity in a pig manure storage pit using small subunit rDNA sequence analysis. FEMS Microbiol. Ecol., 52: 229-242.

Tang, J., T. Kanamori, Y. Inoue, T. Yasuta, S. Yoshida and A. Katayama (2004) Changes in the microbial community structure during thermophilic composting of manure as detected by the quinone profile method. Process Biochem., 39: 1999-2006.

Thummes, K., J. Schäfer, P. Kämpfer and U. Jäckel (2007) Thermophilic methanogenic Archaea in compost material: occurrence, persistence and possible mechanisms for their distribution to other environments. Syst. Appl. Microbiol., 30: 634-643.

Yamada, T., A. Suzuki, H. Ueda, Y. Ueda, K. Miyauchi and G. Endo (2008) Successions of bacterial community in composting cow dung wastes with or without hyperthermophilic pre-treatment. Appl. Microbiol. Biotechnol., 81: 71-81.

Yamamoto, N., K. Otawa and Y. Nakai (2009) Bacterial communities developing during composting processes in animal manure treatment facilities. Asian-Aus. J. Ani. Sci., 22: 900-905.

Yamamoto, N., K. Otawa and Y. Nakai (2010) Diversity and abundance of ammonia-oxidizing bacteria and ammonia-oxidizing archaea during cattle manure composting. Microb. Ecol., 60: 807-815.

Development of a High-Efficiency Methane Fermentation Process for Hardly Degradable Rice Straw

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Abstract

In order to develop the new and high-efficiency methane fermentation process for hardly degradable rice straw, the co-digestion performance of different amount of rice straw and sewage sludge was investigated. The digestion of rice straw and sewage sludge under the investigated condition resulted in VFA (volatile fatty acid) accumulated in the digester. The influences of temperature, moisture content, the ratio of carbon to nitrogen (C:N ratio) and initial pH value on VFA accumulation in the hydrolysis step of methane fermentation were tested. The results showed that the total VFA concentration was increased with initial pH value and moisture content, and was decreased with temperature and the ratio of carbon to nitrogen. Then the feasibility of rice straw pretreatment with the accumulated VFA from the fermentation process was studied, and 10.7% of weight loss was obtained by pretreatment with drip washing VFA solution. The production of methane gas from rice straw was significantly enhanced as nearly as two times by drip washing VFA solution pretreatment than that of untreated rice straw. This suggested that the accumulated VFA from the rice straw fermentation process can be used as a kind of pretreatment reagent for improving biodegradability and methane production. So a low-cost and high-efficiency methane fermentation process for hardly degradable lignocellulosic materials such as rice straw could be established.

Introduction

Anaerobic digestion has become one of the major treatment techniques for municipal sewage sludge and manure. In many cases of the world, methane recovered from digestion supplied sufficient energy to support wastewater treatment plants and farms. And in fact, methane generated from organic wastes has already provided all daily energy requirements for residences in some villages in China (Cui and Xie, 1985). Despite all of advantages of anaerobic digestion process, however, major obstacles still remain to be resolved for the practical application of methane fermentation for an important group of waste --- the lignocellulosic materials, which include forestry products, agricultural crop residues, and urban refuse. Anaerobic digestion of these materials is often a slow and frequently incomplete process, which in most cases makes methane production from these materials uneconomical at current energy prices, because these materials are predominantly composed of various polymers which include cellulose, hemicellulose and lignin in chemical which intimately interconnected and form the complex lignocellulosic structure. Besides this, other factors which are also important in methane production include acidification (Hill et al., 1987), ammonia concentration (Sung and Liu, 2003; Hansen, K.H., 1998), digestion conditions (Zennaki et al., 1996; Sharma et al., 1988; Bardiya and Gaur, 1997; Sundrarajan et al., 1997; Singh et al, 1995), and the nutritional requirements of microbes (Kayhanian and Rich, 1995). However, how to enhance the gas production rate from lignocellulosic material and reduce the digestion failure resulting from the acid accumulation or ammonia inhibition are poorly understood.

Since lignocellulosic materials include literally thousands of different plant species and crop residues, it is by no means that this research would be able to deal with every single material. Several representative lignocellulosic materials were therefore selected

for detailed study (Forster-Carneiro, 2007; Vedrenne, 2008; Ting, 2007; Sosnowski, 2003). The criteria of selection were twofold: first, materials representing the broad range of characteristics of lignocellulosic materials were desired so that the influence of material property on biodegradation could be investigated; second, materials having good potential as feed stocks for methane production at commercial scale were sought.

On the other hand, biological wastewater treatment has been used widely in the world, but large amounts of sewage sludge are produced in this process. Rapid urbanization in many areas of the world has resulted in a drastic increase of sewage sludge with a typical person generating over 50 g of dry solids every day (Hudson, 1995). In China alone, about 0.88–1.55 million tons of dry sludge was produced in 2003 (Liu, 2008). The treatment and disposal of sludge have become one of the most important and complex problems. In fact, the main part of sewage sludge is organics (biomass) and it can become a source of energy (e.g., methane). The main component of sewage sludge is also microbial cells, so that it can be used as the inoculum of the fermentation. So in the present study, rice straw and sewage sludge was selected as the substrate of the fermentation.

Anaerobic digestion of lignocellulosic materials is a very complex process, not only because of bacteria diversity and process complexity, but also because of the nature of the complex polymeric structure of the materials themselves. Previous studies have focused on various aspects of the digestion process. The aim of this research is to optimize the fermentation conditions of acid accumulation and to discuss the fermentation effect of materials pretreated by the drip washing solution which obtained from acid fermentation process. Then, the results obtained from this and other research are applied to the appropriate design of a new high-efficiency methane fermentation process for methane production from lignocellulosic materials.

Materials and methods

Fermentation of rice straw

Rice straw used in this study was collected from field near Tsukuba (Ibaraki, Japan) in 2008. The air-dried rice straw was cut into small pieces and further milled to powder. The particles having a size between 40-mesh (0.45mm) and 50-mesh (0.36mm) were stored in plastic bag at room temperature until further processed. The sewage sludge (TS=1.77%, VS=79.5%TS) from Kasumigaura sewer office (Ibaraki, Japan) was used as original seed methanogens sludge. It was kept in the refrigerator at 4˚C before the fermentation experiment.

The batch fermentation experiment was conducted with rice straw and sewage sludge. Fermentor bottles (500 mL, SIBATA) were used as batch fermentation reactors. 148 g sewage sludge was added into the bottle along with 14 g, 26 g and 40 g of rice straw, respectively. Fermentation of sewage sludge only was used as the control. These two materials were mixed and the pH value of mixture was adjusted to 7 by sodium hydroxide. The bottles were stopped with silica gel stoppers, which were air impermeable even after repeated sampling with syringes. All of bottles were initially flushed with nitrogen gas prior to fermentation test, and then they were incubated at 35˚C for 30 days. Gas production and composition from each bottle were monitored periodically every 3 days. Sampling was conducted in a glove-box (COY Laboratory Products, USA).

Acidogenic fermentation

The composition of rice straw was as follows: total solids (%, w/w) 92.2, volatile solids (%, TS) 81.9, carbon (%, dwb) 35.11, hydrogen (%, dwb) 5.47, nitrogen (%, dwb) 0.87, where dwb means dry weight basis. The sewage sludge was obtained from Kasumigaura sewer office (Ibaraki, Japan) and used as inoculum. Its composition was as follows: total solids (%, w/w) 1.77, volatile solids (%, TS) 79.5, carbon (%, dwb) 36.36, hydrogen (%, dwb) 4.10, nitrogen (%, dwb) 5.76.

Four batch fermentation experiments were conducted to assess the fermentability of the rice straw and sewage sludge to determine the amount of organic matters that could be converted into VFA. Fermentor bottles (500 mL, SIBATA) were used as batch fermentation reactors. Here, sewage sludge was mixed with rice straw by the ratio of 1:3.8 and added into the bottle. Then the pH value of mixture was adjusted to 6, 7, and 8 by sodium hydroxide, respectively. All of bottles were initially flushed with nitrogen gas prior to fermentation test, and then they were incubated at 35˚C or 55˚C for 30 days.

Preparation of drip washing solution

In order to obtain drip washing solution, batch acidogenic fermentation experiment with rice straw (<60-mesh) and sewage sludge (w/w=1:3.8) was conducted at 35˚C. After the pH value of sludge was adjusted to 7.0 by sodium hydroxide, it was mixed with rice straw and then fed into the fermentor bottles (500 mL, SIBATA). Then the fermentors were flushed with nitrogen gas for 2 min to exclude oxygen. The fermentation was operated at 3 days HRT because the results of preliminary experiment showed that the acid concentration reached the maximum value at the third day (data was not shown). For the production of the drip washing solution, distilled water was added into fermentors and kept for 1 h at room temperature. Then the liquid fraction (drip washing solution) was subsequently separated from solid material by screening filter (60-mesh). The drip washing solution was kept at 4˚C in the refrigerator for the anaerobic digestion of rice straw.

Analyses

Both rice straw and sewage sludge contain varying amounts of water. The oven-drying method was used to determine their moisture content (or total solid). A certain amount of sample were weighed and placed in a porcelain crucible, and then the sample (with stopper removed) was placed in a 105˚C oven (NRG-1400, Nihon Freezer Co., Ltd.) and dried to constant weight overnight. The sample was then placed in a desiccator for cooling and then weighed again. The moisture content, represented by the loss in weight at 105˚C, is reported as percentage of the original sample weight. Analyses were performed in triplicate. The air-dried sample of rice straw and sewage sludge was grounded into the powder and their carbon and nitrogen content was analyzed by element analyzer (Perkin Elmer JST 2400).

The ash content is a measure of the inorganics present in the materials. The analysis was carried out using porcelain crucibles. The sample was first dried and weighed, and then fired in a 600˚C-muffle furnace (Fuji Electronics Industry Co., Ltd.) overnight. The final residual remaining in the crucible is reported as the ash content (dry weight basis). The lost weight is reported as the volatile solid.

Biogas analyses of fermentor headspace were performed using a chromatography (GC-8A, SHIMAZU, Japan) equipped with a thermal conductivity detector (80˚C) and a Porapak Q column (60˚C). Nitrogen gas was used as carrier gas at a flow rate of 1 mL/min. Certified gas standards were employed for the standardization of hydrogen, methane and carbon dioxide. Standards were run on each day that samples were measured. Each sample was analyzed at least twice, and a 1 mL volume of gas was injected for analysis.

About 1 g of substrate was taken out from the fermentor and was added into 10 mL of distilled water. The mixture was then centrifuged in a centrifugal machine. The supernatant was quantified by HPLC (Jasco International Co., Japan), equipped with UV/VIS detectors after filtrated by 0.45 μm membrane. Formic acid, acetic acid and propionic acid in the samples were determined by a Inertsil ODS-4 column at 40˚C. 0.05 mol/L of ammonium dihydrogen phosphate solution (pH=2.4) was used as eluent at a flow rate of 1.0 mL/min.

Results and discussion

Performance of rice straw and sewage sludge anaerobic digestion

The chemical composition of the mixture of rice straw and sewage sludge is given by Table 1. The amount of sewage sludge in four fermentors is same, while the addition amount of rice straw is 14, 26, and 40 g, respectively. According to the calculation, the

Table 1. Chemical composition of mixture of rice straw and sewage sludge.

	Fermentor NO.1	Fermentor NO.2	Fermentor NO.3	Fermentor NO.4
Rice straw (g)	0	14	26	40
Sewage sludge (g)	148	148	148	148
The initial pH value	7.0	7.0	7.0	7.0
Moisture content (%)	98.2	90.0	85.0	80.0
C:N ratio	6.3	21	26	30

moisture content and ratio of C: N are in the range of 98.2% to 80.0% and 6.3 to 30, respectively. Because the sewage sludge was acidic, and its pH value is 6.4, the initial value of pH of the substrate was adjusted to 7 by sodium hydroxide.

Fig. 1 showed the methane production of different fermentors. In 30 days, sewage sludge (fermentor NO.1) produced methane gas continuously and the total volume of methane was 270 mL. But with the addition of rice straw (fermentor NO.2, NO.3 and NO.4), the cumulative methane production of substrate in these four fermentors was decreased gradually. At the end of the fermentation process, they were achieved 64, 27, and 12 mL for the addition of 14, 26, and 40 g rice straw, respectively. It is obviously that no methane gas was released from the degradation of the rice straw, and it can be presumed that the addition of rice straw has negative effect on the production of the methane gas.

Fig. 2 showed the biogas production of different fermentors. Similar to methane production, sewage sludge produced biogas continuously in the whole fermentation process, and its total volume achieved 487 mL. In the contrary case, gas production with rice straw addition is mainly concentrated in the first 10 days. The cumulative biogas production was 397, 755, and 880 mL, respectively. Comparison with biogas production of sewage sludge, the production of

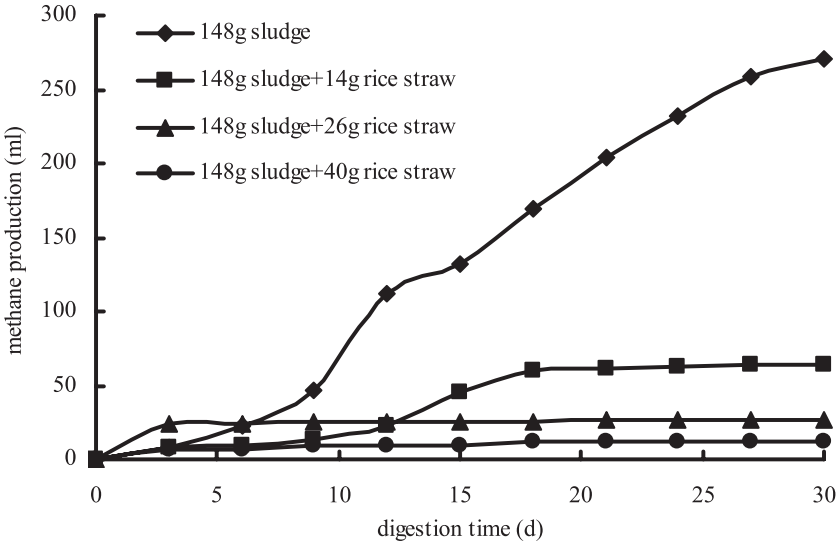

Fig. 1. Cumulative methane production with different rice straw addition.

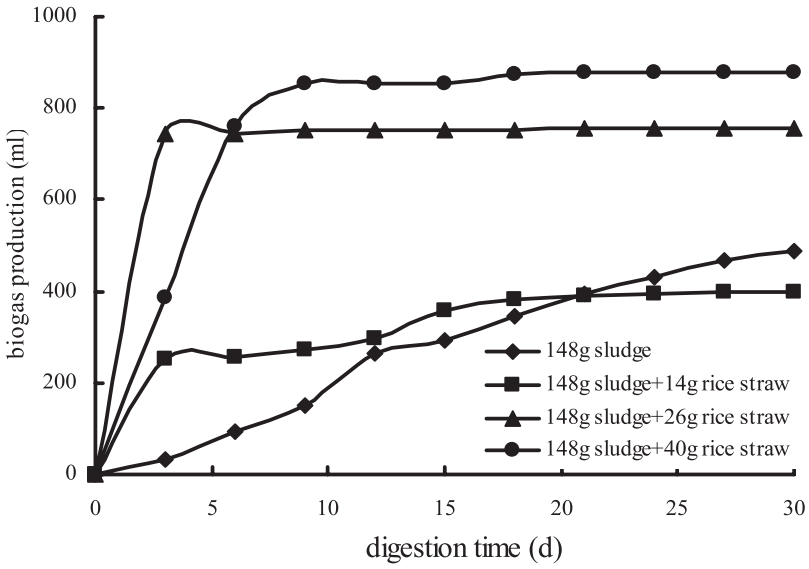

Fig. 2. Cumulative biogas production with different rice straw addition.

rice straw addition increased. This indicated that rice straw was also degraded in the fermentation process, and released a certain volume of biogas. The more addition amount of rice straw was added, the more biogas was produced. But combined with the result of methane production, the majority of the biogas produced from rice straw should be hydrogen and carbon dioxide. It is well-known that the first step of the degradation of rice straw is hydrolysis, and the main product is hydrogen. Therefore, large quantity of the hydrogen was released in the earlier stage, and the carbon dioxide was released in the later stage.

Figs. 3, 4, 5 and 6 showed the component of the biogas in each experiment. For the fermentation of sewage sludge, no hydrogen gas was produced in the whole fermentation process, and the methane gas accounted for 70% of the biogas. For the 16 g addition of rice straw, the hydrogen gas could be detected just in the first 3 days, and the methane gas could also be detected in the whole process while its percentage just achieved 57%. For the 24 g addition of rice straw, hydrogen, methane and carbon dioxide was exist from the beginning to the end. The highest value of hydrogen was 46% on the third day and the methane achieved 39% at the end of the fermentation. For the 40 g addition of rice straw, hydrogen gas accounted for 27% at the end of the fermentation and the methane gas was not higher than 20% in the whole process.

Fig. 7 showed the change of pH value of four fermentors in the fermentation process. The pH value of sludge fermentation was maintained at 7.4 while three others were maintained at 4.7, 5.2, and 5.5 at the end of fermentation process, respectively. This

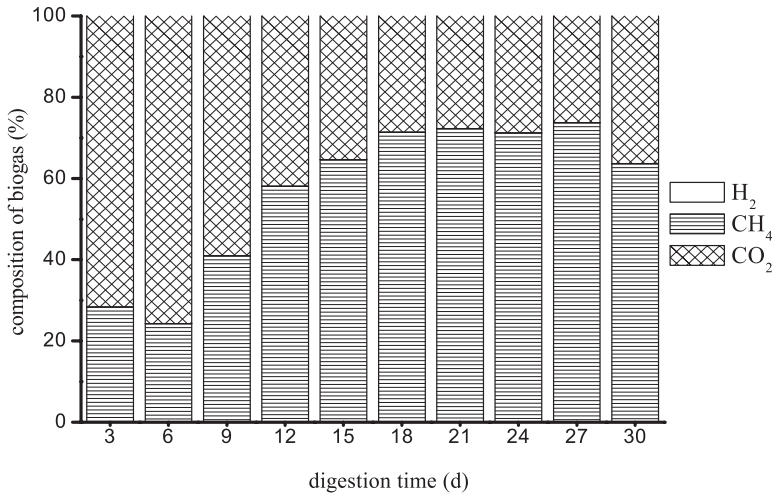

Fig. 3. Composition of biogas produced in fermentor NO.1.

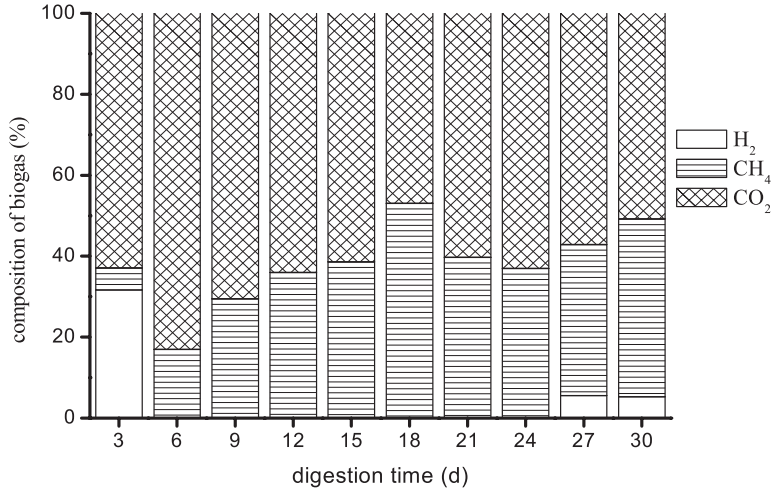

Fig. 4. Composition of biogas produced in fermentor NO.2.

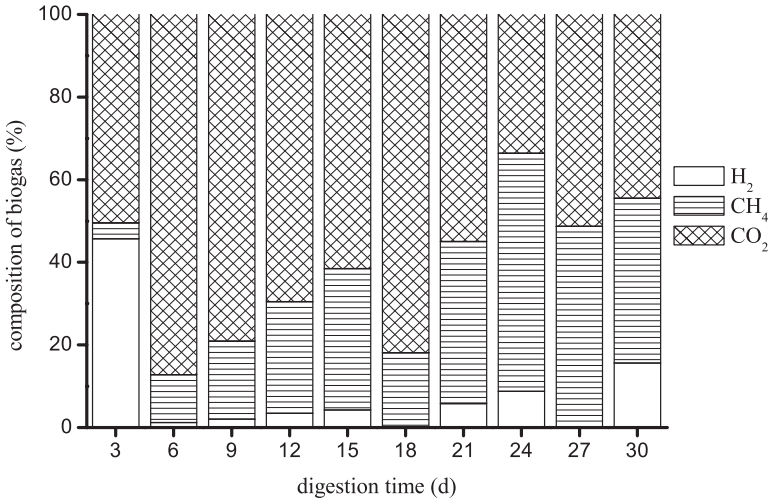

Fig. 5. Composition of biogas produced in fermentor NO.3.

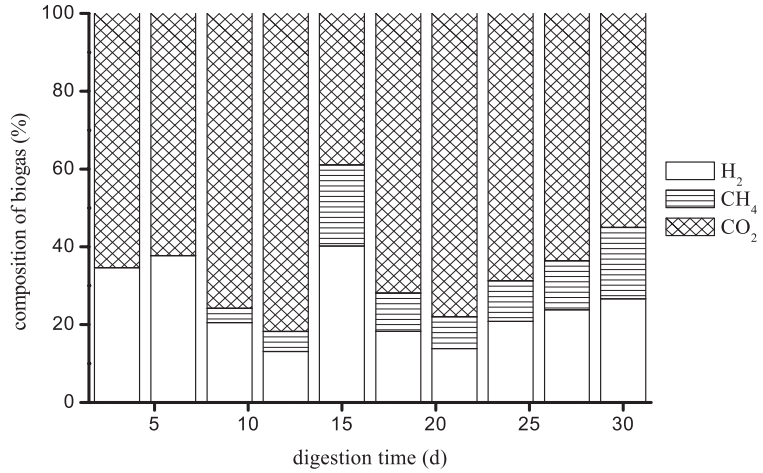

Fig. 6. Composition of biogas produced in fermentor NO.4.

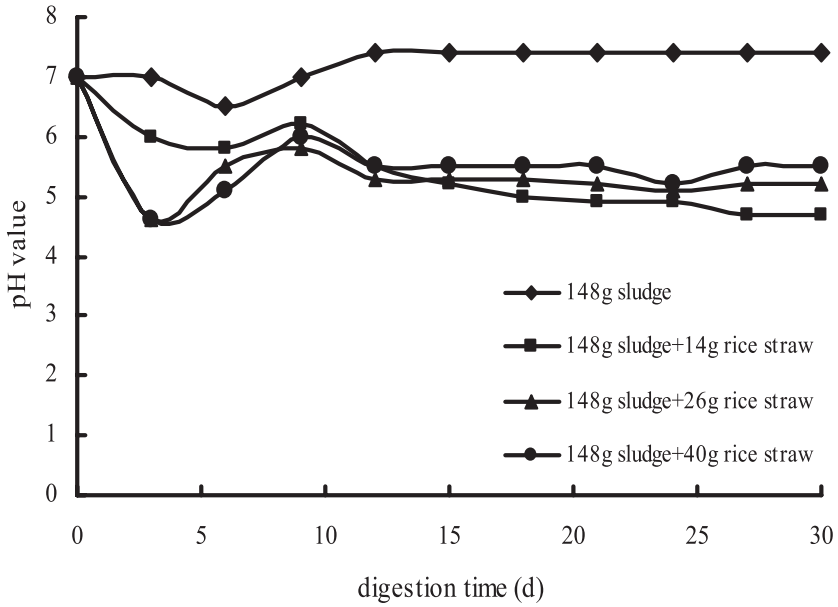

Fig. 7. Change of pH in the fermentation process.

resulted from the production of volatile fatty acid which generated in the hydrolysis of rice straw. It means that acidification was occurred in the fermentor NO.2, NO.3 and NO.4, the degradation of rice straw resulted in the acidification of the fermentation system.

The co-digestion of sewage sludge and different amount of rice straw indicated that digestion of sewage sludge produced methane gas continuously while the addition of rice straw decreased the methane production but increased the hydrogen production. The degradation of rice straw made contributions to the production of VFA and hydrogen.

Acidogenic fermentation of rice straw and sewage sludge

Based on the presented study, three major factors which affect the methane fermentation with rice straw and sewage were chosen to be studied here: (1) temperature, (2) initial pH value, (3) moisture content, (4) C:N ratio. A better understanding of how these factors control the hydrolysis of rice straw and the VFA production should improve knowledge about the mechanisms of lignocellulosic degradation, the design of methane fermentor, and the understanding of best environmental conditions for reactor operation. In order to find out the optimal conditions for the acid accumulation and to obtain the drip washing solution with concentration as high as possible, acidogenic fermentation of rice straw and sewage sludge was carried out.

The effect of temperature on acidogenesis of rice straw and sewage sludge was showed in Table 2. For the acidogenic fermentation at 35˚C, acetic, propionic and butyric acid had been detected in the fermentor. The concentration of acetic acid was increased continuously while that of propionic and butyric acid had no obvious change from the third day. The highest value of these three acids was 18525, 15315 and 10356 ppm, respectively. The TVFA achieved its highest value of 44197 ppm on the sixth day, and the acetic acid accounted for the main content.

For the acidogenic fermentation at 55˚C, besides acetic, propionic and butyric acid, formic acid had also been detected in the fermentor. The concentration of formic and butyric acid had no obvious change in the whole fermentation process, while the acetic acid concentration was continuously increased and that of propionic acid was decreased. The TVFA concentration achieved its highest value of 42312 ppm on the seventh day. At that time, acetic acid accounted for the maximum proportion of 41.6%, then was propionic and butyric acid which accounted for 22.8% and 23.7%, respectively, and the least one was formic acid accounted for 12.0%.

Compare this two fermentor's performance, the concentration of acetic acid and butyric acid was nearly same but that of propionic acid at 35˚C was much higher than that at 55˚C. That is to say, more acid was produced at 35˚C. Therefore, 35˚C is suitable for the VFA accumulation. This coincides with the VFA distribution observed by Penaud et al. (1997) who reported a change in VFA composition due to temperature and, more specifically, higher percentages of propionate at lower temperature.

Effect of initial pH value on acidogenesis of rice straw and sewage sludge was presented in Table 3. The acid products of acidogenic fermentation at initial pH of 6 were as follows: formic, acetic, propionic and butyric acid. The highest value of acid concentration was obtained on the sixth day, and they were 4312, 13703, 13638, and 11120 ppm, respectively. The TVFA concentration was achieved 42775 ppm on that day.

Table 2. Effect of temperature on acidogenesis of rice straw and sewage sludge.

	Temperature (˚C)	
	35	55
TVFA (ppm)	44197	42312
Formic acid (ppm)	---	5084 (12.0%)
Acetic acid (ppm)	18524 (41.9%)	17583 (41.6%)
Propionic acid (ppm)	15315 (34.7%)	9631 (22.8%)
Butyric acid (ppm)	10356 (23.4%)	10013 (23.7%)

The acids produced at the initial pH of 8 were just acetic, propionic, and butyric acid, formic acid had not been detected. At pH of 8, TVFA concentration was 49123 ppm on the fourth day, which was the highest value obtained in the whole fermentation period. The highest value of acetic, propionic and butyric acid was 21519, 15581, 12024 ppm, respectively.

For the different initial pH value, the acid concentration increased with the pH value increased. In particularly, acetic acid concentration increased sharply. The concentration of propionic acid and butyric acid had a slightly increase with the increase of the pH value. In totally, the total concentration of VFA increased as the pH value increased. This is coincided with the other reseachers such as Lim (2008) and Bengtsson (2008) that high pH value is benefit to the production of VFA.

Effect of moisture content on acidogenesis of rice straw and sewage sludge was presented in Table 4. For these five experiments, acetic, propionic and butyric acid were detected. The highest value of TVFA at 70% moisture content was achieved 22367 ppm at sixth day, and the VFA speciation results indicate that acetic and butyric were the most prevalent VFA generated throughout the experiment.

For other four experiments, the highest value of VFA at 75%, 80%, 85% and 90% moisture content were 20178, 13408, 8508, and 6939 ppm, respectively. Acetic and butyric acid were also the most prevalent VFA generated.

According to above results, it can be concluded that TVFA concentration increased with increasing

Table 3. Effect of initial pH value on acidogenesis of rice straw and sewage sludge.

	pH		
	6	7	8
TVFA (ppm)	42775	44197	49123
Formic acid (ppm)	4312 (10.1%)	---	---
Acetic acid (ppm)	13703 (32.0%)	18524 (41.9%)	21519 (43.8%)
Propionic acid (ppm)	13638 (31.9%)	15315 (34.7%)	15581 (31.7%)
Butyric acid (ppm)	11120 (26.0%)	10356 (23.4%)	12024 (24.5%)

Table 4. Effect of moisture content on acidogenesis of rice straw and sewage sludge.

	Moisture content (%)				
	70	75	80	85	90
TVFA (ppm)	22367	20178	13408	8508	6939
Acetic acid (ppm)	6932 (31.0%)	6324 (31.3%)	4221 (31.5%)	2978 (35.0%)	2367 (34.1%)
Propionic acid (ppm)	3682 (16.5%)	2930 (14.5%)	2510 (18.7%)	2059 (24.2%)	1199 (17.3%)
Butyric acid (ppm)	11752 (52.5%)	10925 (54.1%)	6677 (49.8%)	3472 (40.8%)	3374 (48.6%)

moisture content. This suggests that low moisture content is benefit for the acid accumulation. It may be explained by the fact that low moisture content has bad fluidity, and it is easily result in partial acidification in the fermentor. On the contrary, the moisture in substrate will promote the uniform distribution of the acids so that the acid concentration was decreased.

Table 5 showed the effect of C:N ratio on acidogenesis fermentation. For these three experiments, the VFAs identified include acetic, propionic, and butyric acid. The highest value of VFA was achieved 19841 ppm at C:N ratio of 10, while that of other two conditions were 18042 ppm at C:N ratio of 20 and 11999 ppm at C:N ratio of 30. Acetic was the most prevalent VFA generated for all of three experiments. The results showed that the VFA concentration decreased with the increasing C:N ratio. The higher VFA concentration was achieved at low C:N ratio.

Methane fermentation of rice straw pretreated by drip washing solution

In experiment, 200 ml drip washing solution was obtained. The HPLC results showed that the acid components in drip washing solution was mainly composed of four kinds of organic weak acids, formic acid, acetic acid, propionic acid and butyric acid. Its concentrations were 0.21, 9.43, 1.27, 0.53 g/L, respectively. It was obvious that the concentration of acetic acid is much higher than other components. Acetic acid was the main acid part of the drip washing solution. The rice straw was then pretreated by actual drip washing solution which obtained from an acidification fermentor for 2 h at 1:20 of solid to liquid ratio. The weight loss was achieved 10.7%.

Methane production from rice straw which is untreated and pretreated by actual drip washing solution was carried out at 35˚C for 30 days. The fermentation of sewage sludge was also conducted as control under the same condition. Fig. 8 showed methane productivity of the fermentors with addition of 1.0 g untreated or pretreated dry rice straw. The data were averages of three replicates. The final methane productivity of pretreated rice straw achieved 1.21$l_{ch4}/l_{fermentor}\cdot d$ while that of untreated rice straw was just 1.13$l_{ch4}/$

Table 5. Effect of C:N ratio on acidogenesis of rice straw and sewage sludge.

	C:N ratio		
	10	20	30
TVFA (ppm)	19841	18042	11999
Acetic acid (ppm, %)	13703, 71.4	7976, 44.2	6614, 55.1
Propionic acid (ppm, %)	3913, 19.7	4569, 25.3	2731, 22.8
Butyric acid (ppm, %)	1764, 8.9	5497, 30.5	2654, 22.1

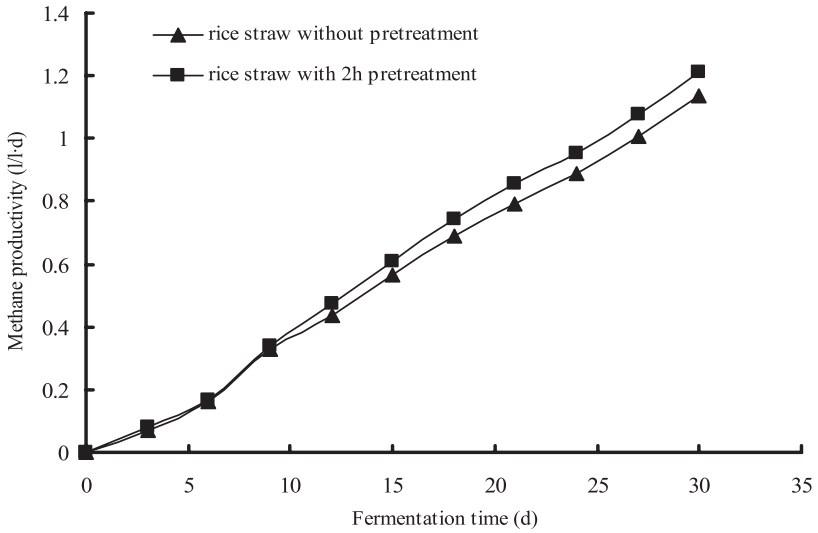

Fig. 8. Methane productivity of rice straw pretreated by drip washing solution.

$l_{fermentor} \cdot d$. Before 12 days, the methane production of two fermentors was almost same while after that there is a marked increase in the methane volume of the pretreated substrate than that of untreated rice straw. The methane gas released in former 12 days perhaps mainly come from the degradation of volatile organic compound in the sewage sludge because it is relatively easy degraded in the comparison with rice straw. On the other hand, methanogens could rapidly increase in this period for microbial degradation of the rice straw in the next step. From the 12th day, the methane productivity of these two substrates shows different. Table 6 presented total gas volume evolved from sewage sludge and 1 g of rice straw with and without pretreatment. For the untreated rice straw, the total methane production of 1 g of pretreated sample was just 73 mL until the 30th day, while the final the methane production of acid pretreated rice straw was increased up to 154 mL. The total biogas volume was also increased from 106 mL to 227 mL after pretreatment. Compared to untreated rice straw, the methane gas production of pretreated rice straw was increased as nearly as two times. The difference between the methane production and biogas production of untreated and pretreated rice straw was evaluated by ANOVA using Microsoft Excel, and the result showed that both methane gas production and biogas production of pretreated samples was significantly ($p \leq 0.05$) higher than that of untreated one. The present results showed that the drip washing solution pretreatment was a beneficial as a method to improve the anaerobic biodegradability of biomass.

Conclusions

The digestion of sewage sludge produced methane gas continuously while the addition of rice straw decreased the methane production but increased the hydrogen production. It is clear that digestion of rice straw and sewage sludge under the investigated condition resulted in the acidification more easily. The degradation of rice straw made contributions to the production of VFA and hydrogen.

For the pretreatment by actual drip washing solution obtained from an acidified methane fermentation process, 10.7% of weight loss was achieved. The production of methane gas from rice straw was enhanced as nearly as two times by pretreatment. This also illustrated that actual dripping solution with low concentration can be used as a kind of pretreatment method for improving biodegradability of rice straw.

In the hydrolysis step of methane fermentation with rice straw and sewage sludge, was increased with initial pH value and moisture content, and was decreased with temperature and the ratio of carbon to nitrogen. The increase of temperature mainly resulted in the decrease of propionic acid, and the increase of pH value mainly due to the increase of acetic acid. Higher moisture content resulted in the higher percentage of butyric, while higher C:N ratio led to higher percentage of acetic acid. The optimal conditions for VFA accumulation were 35˚C, initial pH of 8, moisture content of 70% and C:N ratio of 10.

This research has attempted to investigate the acidification performance of methane fermentation, and tried to enhance methane productivity from hardly degradable rice straw by using drip washing VFA solution obtained from an acidified methane fermentation system. It could be possible to develop a low-cost and high-efficiency methane fermentation process for hardly degradable rice straw.

References

Bardiya, N. and A. C. Gaur (1997) Effects of carbon

Table 6. Total gas volume evolved from sewage sludge and 1 g of rice straw.

	CH_4 production (mL)	biogas production (mL)
Sewage sludge	1297±16	1909±12
Sewage sludge + untreated straw	1370±15	2015±18
Sewage sludge + pretreated straw	1451±20	2136±18

and nitrogen ratio on rice straw biomethanation. J. Rural Energy, 4 (1-4): 1-16.

Bengtsson, S., J., Hallquist, A., Werker and T. Welander (2008) Acidogenic fermentation of industrial wastewaters: Effects of chemostat retention time and pH on volatile fatty acids production. Biochem. Eng. J., 40: 492-499.

Cui, X. and Z. Xie (1985) "An outline on the biogas development in China." In Anaerobic Digestion, Proceedings of Fourth International Symposium on Anaerobic Digestion, Guangzhou, China, Nov. 11-15.

Forster-Carneiro, T., M., Pérez and L.I. Romero (2007) Composting potential of different inoculum sources in the modified SEBAC system treatment of municipal solid wastes. Bioresour.Technol., 98: 3354-3336.

Hansen, K. H., I., Angelidaki and B. K. Ahring (1998) Anaerobic digestion of swine manure: inhibition by ammonia. Wat. Res., 32: 5-12.

Hill, D. T., S. A., Cobb and J. P. Bolte (1987) Using volatile fatty acid relationships to predict anaerobic digester failure. American Society of Agricultural Engineers, 30: 496-501.

Hudson, J. A. (1995) Treatment and disposal of sewage sludge in mid-1990s. J. Chart. Inst. Water Environ. Manag., 9: 93-100.

Kayhanian, M. and D. Rich (1995) Pilot-scale high solids thermophilic anaerobic digestion of municipal solid waste with an emphasis on nutrient requirements. Biomass Bioenergy, 8 (6): 433-444.

Lim, S. J., B. J., Kim, C. M., Jeong, J., Choi, Y. H., Ahn and H. N. Chang (2008) Anaerobic organic acid production of food waste in once-a-day feeding and drawing-off bioreactor. Bioresour. Technol., 99: 7866-7874.

Liu X., H., Liu, J., Chen, G., Du and J.Chen (2008). Enhancement of solubilization and acidification of waste activated sludge by pretreatment. Waste Manage. 28: 2614-2622.

Penaud, V., J. P., Delgenes, M., Torrijos, R., Moletta, B., Vanhoutte and P. Cans (1997) Definition of optimal conditions for the hydrolysis and acidogenesis of a pharmaceutical microbial biomass. Process Biochem., 32 (6): 515-521.

Sharma, S. K., I. M., Mishra, M. P., Sharma and J. S. Saini (1988) Effect of particle size on biogas generation from biomass residues. Biomass, 17: 251-263.

Singh, L., M. S., Maurya, K. V., Ramana, S. I., Alam and L. Singh (1995) Production of biogas from night soil at psychrophilic temperature. Bioresour. Technol., 53 (2): 147–149.

Sosnowski, P., A., Wieczorek and S. Ledakowicz (2003) Anaerobic co-digestion of sewage sludge and organic fraction of municipal solid wastes. Advances in Environmental Research, 7: 609-616.

Sundrarajan, R., A., Jayanthi and R. lango (1997) Anaerobic digestion of organic fractions of municipal solid waste and domestic sewage of Coimbatore. Indian J. Environ. Health, 39(3): 193-196.

Sung, S. and T. Liu (2003) Ammonia inhibition on thermophilic anaerobic digestion. Chemosphere, 53: 43-52.

Ting, C. H. and D. J. Lee (2007) Production of hydrogen and methane from wastewater sludge using anaerobic fermentation. Int. J. Hydrogen Energy., 32: 677-682.

Vedrenne, F., F., Béline, P., Dabert and N. Bernet (2008) The effect of incubation conditions on the laboratory measurement of the methane producing capacity of livestock wastes. Bioresour.Technol., 99, 146-155.

Zennaki, B. Z., A., Zadi, H., Lamini, M., Aubinear and M. Boulif (1996) Methane Fermentation of cattle manure: effects of HRT, temperature & substrate concentration. Tropicultural, 14(4): 134-140.

How Does Differential Timing of Grazing Affect Plant Species Diversity in Improved and Semi-Natural Pastures?

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Abstract

The succession–disturbance balance determines the present successional stage. Therefore, differential timing of grazing is expected to create species-diverse pasture at the landscape scale because various stages of succession will coexist. Here, we examined how differential timing of grazing affects the species diversity of vegetation in three representative types of Japanese pastures (improved, partially improved semi-natural, and semi-natural). We simulated the effect of grazing in patches during three months and recorded the percentage cover of each plant species at 1, 3, and 4 months after grazing. We calculated diversity indices using these community data while varying the sampling probabilities. Estimated diversity was greater with differential timing of grazing than with contemporary grazing in the improved pasture, but diversity was lower in the semi-natural pasture, possibly because directional succession following grazing occurred only in the improved pasture. Our findings indicate that differential timing of grazing should be used for biodiversity management only in landscapes in which the vegetation communities show directional succession after grazing.

Introduction

The original concept of succession following disturbance emphasized the directional replacement of certain types of species occurring over time in a particular order (McCook 1994; Platt & Connell 2003). Disturbance tends to either delay succession or reverse the community to the initial state of succession. Thus, differential timing of disturbances within a landscape is expected to create a species-diverse landscape (shifting mosaic) because various stages of succession will coexist (Olff & Ritchie 1998; Yoshihara et al. 2010). A grazing study by Smith et al. (2000) demonstrated this concept; they found a larger increase in plant diversity with the combination of spring and autumn grazing than with either spring or autumn grazing alone.

In temperate pastures, plant succession in grazed areas generally shifts from herbs to grasses to woody plants or trees (Balmer & Erhardt 2000). However, the amount of seed rain and facilitating or inhibiting effects among plants influence the succession rate and/or succession pathway (Vieira et al. 1994; Pakeman & Small 2005). In an acid grassland, for example, plots disturbed in winter (i.e., less seed rain) had significantly greater cover of perennial forbs and slower rates of revegetation than those disturbed in summer (more seed rain) (Pakeman & Small 2005). Therefore, the effect of grazing timing on the species diversity of vegetation would be expected to depend on grassland type.

The typical vegetation in a Japanese grazing pasture is artificially sown, which augments the existing pasture vegetation and thus improves its productivity. However, creating and maintaining this improved pasture require large amounts of fertilizer, exotic grass seeds, and higher costs for regular sward renovation. Thus, in recent years grazing semi-natural pastures that support various wild plant species has received special attention as an alternative sustainable grazing system (Isselstein et al. 2005; Sato 2005).

Our objective is to develop a grazing system that will increase biodiversity in several types of Japanese pastures that vary in community assemblage. In the present study, we compared the expected species diversity of vegetation under simulated contemporary

grazing versus grazing with differential timing, and we examined the effect of grazing timing on diversity patterns in improved, partially improved, and semi-natural pastures.

Simulating Cattle Grazing

We conducted our study at the Kawatabi Research Station of Tohoku University, 60 km north of Sendai, Japan (38˚44'N, 140˚45'E, 300–600 m above sea level). The climate in this region is temperate, with a mean annual temperature of 10.2˚C and a mean annual rainfall of 1660 mm, based on records from 1979 to 2000. The landscape is characterized by semi-natural pastureland surrounded by secondary forests. The soils are derived from acidic volcanic ash and have adequate supplies of humus.

We used three types of pasture at this site: (1) improved pasture, which has been used for rotational grazing by cattle every year from May to early November, is characterized by exotic grasses that are sown every spring after simple ground renovation; (2) partially improved semi-natural pasture has sown exotic grasses and received a single fertilizer application in 2008; (3) semi-natural pasture is mowed or grazed every autumn and receives no sown exotic species or renovation. In April 2009, we erected 10× 10 m fences around four blocks of land in each pasture to prevent cattle from interfering with the treatments. In each fenced exclosure, we created 20-, 40-, 60-, and 100-cm diameter plots of mowed area in mid-May, late June, and early August, sizes and dates that were selected based on observed grazing activities and a practical grazing regime at this site. Twelve 50×50-cm undisturbed plots were also located in each block. We recorded the proportion of each plot covered by each plant species every 2 weeks after disturbance.

To simulate contemporary grazing, we pooled the community data from 48 plots (4 sizes × 3 monrhs × 4 replications) collected 2 months after mowing and compared these data to those from 48 undisturbed plots. To simulate differential timing of grazing, we pooled the community data of 48 mowed plots gathered in early September (i.e., from 1 to 4 months after grazing) and compared these data to those of 48 undisturbed plots.

We sampled 48 plots from the 96 pooled plots in each simulation, and then calculated Shannon's index of diversity ($H = -\Sigma\ P_i \ln P_i$, where P_i is the relative abundance of species i). This process was replicated 1000 times by means of bootstrap iterations, and the average values of diversity were calculated. To predict biodiversity change while varying the proportion of grazing habitat types in the landscape, we resample plots from each habitat type with probability density function p, which varied at intervals of 0.02 from 0.0 to 1.0. These analyses were performed with R software version 2.9.1 (R Development Core Team 2009).

Comparison of the Expected Species Diversity of Vegetation under Simulated Contemporary Grazing Versus Grazing with Differential Timing

In the improved pasture, we found a total of 15, 15, and 16 species in the treatment plots grazed in mid-May, late June, and early August, respectively (Table 1). The most abundant species differed among the plots with different grazing timing. Among the five most abundant species in each plot, *Anthoxanthum odoratum*, *Dactylis glomerata* and *Viola verecunda* were observed only in the plots grazed in May and August, respectively.

In partially improved semi-natural pasture, we found a total of 20, 19, and 20 species in the treatment plots grazed in May, June, and August, respectively (Table 1). In the plots grazed in May and June *Pteridium aquilinum* was the most abundant species, whereas *Agrostis alba* was the most abundant species in plots grazed in August. *Agrostis alba* was not among the top five abundant species in plots grazed in May and June.

In semi-natural pasture, we found a total of 17, 19, and 13 species in the treatment plots grazed in May, June, and August, respectively (Table 1). *Miscanthus sinensis* was one of the most dominant species in all plots. Among the three pasture types, partially improved semi-natural pasture showed the greatest variance in cover of the five most dominant species.

In improved pasture, the estimated diversity index in the differential timing grazing treatment was slightly greater than that in the contemporary grazing treatment across all proportions of grazed area (Fig. 1, top). The difference in diversity between treatments decreased as the proportion of grazed area increased. The partially improved grassland showed little difference in species diversity between the dif-

Table 1. Mean cover and standard deviation (SD) of the five most abundant species in plots of three pasture types grazed at different times

(a) Improved pasture

May			June			August		
Species	cover (%)*	SD	Species	cover (%)	SD	Species	cover (%)	SD
Agrostis alba	11.25	10.4	*Rumex acetosella*	12.19	15.3	*Poa pratensis*	15.00	11.4
Anthoxanthum odoratum	9.38	14.3	*Agrostis alba*	10.00	13.7	*Carex nubigera*	9.06	10.5
Poa pratensis	8.81	12.9	*Poa pratensis*	7.81	8.3	*Viola verecunda*	6.31	8.3
Dactylis glomerata	6.56	12.7	*Trifolium repens*	7.50	17.3	*Rumex acetosella*	5.81	6.9
Carex nubigera	6.44	8.1	*Carex nubigera*	4.38	8.1	*Trifolium repens*	5.81	13.4
Total	68.13	16.0	Total	57.19	36.1	Total	63.75	16.0

(b) Partially improved semi-natural pasture

May			June			August		
Species	cover (%)	SD	Species	cover (%)	SD	Species	cover (%)	SD
Pteridium aquilinum	43.75	31.1	*Pteridium aquilinum*	24.38	25.5	*Agrostis alba*	20.63	35.4
Carex phacota	16.25	15.0	*Anthoxanthum odoratum*	21.56	20.3	*Pteridium aquilinum*	9.69	12.3
Carex sp.	6.25	7.4	*Carex* sp.	7.50	11.1	*Anthoxanthum odoratum*	8.00	9.3
Anthoxanthum odoratum	5.63	7.9	*Rumex acetosella*	6.25	7.1	*Carex* sp.	4.06	10.2
Aster iinumae	5.63	11.5	*Carex phacota*	3.75	6.4	*Rumex acetosella*	3.94	8.6
Total	99.38	38.3	Total	77.94	49.4	Total	62.38	37.1

(c) Semi-natural pasture

May			June			August		
Species	cover (%)	SD	Species	cover (%)	SD	Species	cover %)	SD
Miscanthus sinensis	18.13	26.1	*Petasites japonicus*	6.25	14.0	*Miscanthus sinensis*	9.25	12.8
Carex nubigera	6.88	11.3	*Miscanthus sinensis*	5.00	10.1	*Aster ageratoides*	1.25	3.4
Anthoxanthum odoratum	6.25	9.5	*Anthoxanthum odoratum*	2.50	5.7	*Anthoxanthum odoratum*	0.81	2.5
Rubus parvifolius	3.75	10.2	*Pteridium aquilinum*	2.06	5.4	*Geranium nepalense*	0.69	1.5
Iris sanguinea	1.88	7.5	*Lysimachia japonica*	1.25	3.4	*Potentilla freyniana*	0.63	1.2
Total	46.00	34.6	Total	23.19	29.0	Total	15.19	14.4

*Mean cover was measured in early September, that is, from 1 to 4 months after the plots were mowed.

ferential timing and contemporary grazing treatments (Fig. 1, middle). In contrast to the improved grassland, the diversity index in the differential timing grazing treatment in semi-natural pasture was lower than that in the contemporary grazing treatment (Fig. 1, bottom). In this case, the difference in diversity between treatments become greater as the proportion of grazed area increased.

Discussion

Our simulated grazing study revealed opposite effects of differential timing of grazing on species diversity in improved and semi-natural pastures. In addition, the response of species diversity in partially improved semi-natural pasture was intermediate between the responses in semi-natural and improved pasture, implying that pasture type affects

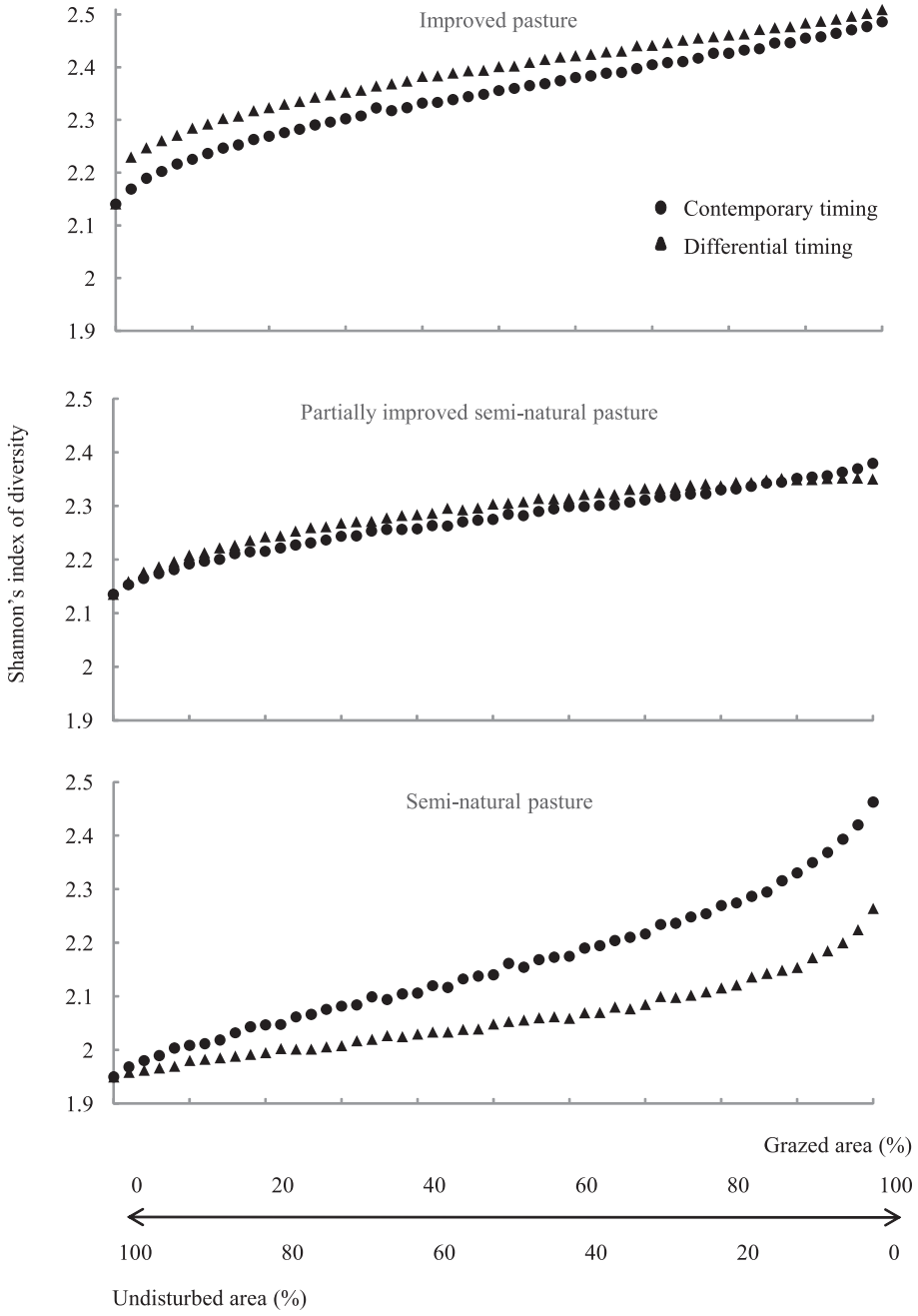

Fig. 1. Expected values of Shannon's index of species diversity after grazing as a function of the proportion of grazed and undisturbed area in three pasture types. Circles and triangles represent, respectively, the average values of diversity based on 1000 iterations in contemporary grazing and differential timing of grazing treatments (see text).

the response. In improved pasture, succession in the grazed patches would occur in the following order: *Poa pratensis*, *Rumex acetosella*, and then *A. alba* communities, which represent the most dominant species recorded 1, 3, and 4 months after grazing, respectively (Table 1). Thus, differential timing of disturbance permits the coexistence of various stages of succession at the pasture scale, leading to an overall increase in species diversity. In semi-natural pasture, however, we did not observe directional replacement of species after grazing; *Miscanthus sinensis* continued to be one of the most dominant species from 1 to 4 months after grazing (Table 1). No positive effects of species diversity by disturbing at various timings seem to be related to this dominance. Instead, site-specific variation in the species composition among contemporary grazed plots (i.e., spatial heterogeneity) may have had a greater effect on species diversity. Indeed, our results showed that the degree of variance from the average percent cover (i.e., coefficient of variance) in semi-natural pasture was relatively high (Table 1), which is probably due to the variation of light availability among microsites in the *M. sinensis* community (Tang et al. 1992; Tang & Washitani 1995). Variation in light availability may also explain why the difference in species diversity between differential and contemporary grazing treatments increased as the proportion of disturbed area increased in semi-natural pasture.

Our results indicate that differential timing of grazing is not always effective in increasing species diversity, because the effect of grazing timing was dependent on the pregrazing vegetation communities. Differential timing of grazing may be a good choice for improved pasture, because after such grazing the communities were clearly separated among successional stages. In semi-natural pasture, however, a single grazing per year may be a better choice in terms of biodiversity management.

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References

Balmer, O. and A. Erhardt (2000) Consequences of succession on extensively grazed grasslands for central European butterfly communities: Rethinking conservation practices. Conserv. Biol., 14: 746-757.

Isselstein, J., B. Jeangros and V. Pavlu (2005) Agronomic aspects of biodiversity targeted management of temperate grasslands in Europe – a review. Agronomy Res., 3: 139-151.

McCook, L. J. (1994) Understanding ecological community succession - causal-models and theories, a review. Vegetatio, 110: 115-147.

Pakeman, R. J. and J. L. Small (2005) The role of the seed bank, seed rain and the timing of disturbance in gap regeneration. J. Veg. Sci., 16: 121-130.

Platt, W. J. and J. H. Connell (2003) Natural disturbances and directional replacement of species. Ecol. Monogr., 73: 507-522.

Olff, H. and M. E. Ritchie (1998) Effects of herbivores on grassland plant diversity. Trends Ecol Evol, 13: 261-265.

R Development Core Team (2009) R: A language and environment for statistical computing.

Sato, S. (2005) Perspectives on the grazing system in Japan. Grassl. Sci., 51: 27-31.

Smith, R. S., R. S. Shiel, D. Millward and P. Corkhill (2000) The interactive effects of management on the productivity and plant community structure of an upland meadow: an 8-year field trial. J. Appl. Ecol., 37: 1029-1043.

Tang, Y., I. Washitani and H. Iwaki (1992) Seasonal variations of microsite light availability within a Miscanthus sinensis canopy. Ecol. Res., 7: 97-106.

Tang Y. and I. Washitani (1995) Characteristics of small-scale heterogeneity in light availability within a Miscanthus sinensis canopy. Ecol. Res., 10: 189-197.

Vieira, I. C. G., C. Uhl and D. Nepstad (1994) The role of the shrub cordia-multispicata cham as a succession facilitator in an abandoned pasture, paragominas, amazonia. Vegetatio, 115: 91-99.

Yoshihara, Y., T. Ohkuro, B. Buuveibaatar, J. Undarmaa and K. Takeuchi (2010) Spatial pattern of grazing affects influence of herbivores on spatial heterogeneity of plants and soils. Oecologia, 162: 427-434.

List of scientific papers in 2010 published by field science group in Graduate School of Agricultural Science, Tohoku University

The Forest – Andisols Group

Amer, S., H. Honma, M. Ikarashi, C. Tada, Y. Fukuda, Y. Suyama and Y. Nakai (2010) *Cryptosporidium* genotypes and subtypes in dairy calves in Egypt. Veterinary Parasitology, 169 (3-4): 382-386.

Fukasawa, Y., T. Osono and H. Takeda (2010) Beech log decomposition by wood-inhabiting fungi in a cool temperate forest floor: a quantitative analysis focused on the decay activity of a dominant basidiomycete Omphalotus guepiniformis. Ecological Research, 25: 959-966.

Fukasawa, Y., T. Osono and H. Takeda (2010) Fungal decomposition of beech coarse woody debris in a temperate forest ecosystem. In: Wood types, properties and uses, L. F. Botannini (Ed.), Nova Science Publishers, New York (Book).

Hasegawa, Y., Y. Suyama and K. Seiwa (2010) Difference in pollen donor composition during the early phases of reproduction as revealed by DNA analysis of pollen grains and seeds in the monoecious tree *Castanea crenata*. In: Single-pollen genotyping, Y. Isagi and Y. Suyama (Eds.), 33-46, Springer, Tokyo (Book).

Heinai, H., T. Ito, S. Nakamura, M. Nanzyo and M. Saigusa (2010) Chemical properties and mineralogical characteristics of tea garden soils in the Cheju Island, Korea. Jpn. J. Soil Sci. Nutr., 81: 1-6 (in Japanese).

Imaji, A. and K. Seiwa (2010) Carbon allocation to defense, storage, and growth in seedlings of two temperate broad-leaved tree species. Oecologia, 162: 273-281.

Imaji, A. and K. Seiwa (2010) Carbon allocation and growth-survival trade-offs in temperate tree species. Journal of Integrated Field Science 7: 9-13.

Ito, T., T. Komiyama, M. Saigusa and M. Morioka (2010) Phosphate composition of swine and poultry manure composts. Jpn. J. Soil Sci. Nutr., 81: 215-223 (in Japanese).

Kanno, H., T. Takahashi and M. Nanzyo (2010) Organic Carbon Accumulation in Andosols: (1) Unaccounted Andosols in Japanese Forest Soil. Journal of Integrated FIeld Science, 7: 65-67.

Kohno, N., K. Yokoyama, M. Saito and T. Marumoto (2010) Effect of terrestrial cyanobacterial mat "Ishikurage" on the growth of Japanese pampas grass in infertile devastated soils. Soil Microorganisms, 64: 3-10.

Komiyama, T., T. Ito and M. Saigusa (2010) Water solubility of phosphorus in animal manure compost. 19th World Congress of Soil Science, Brisbane, Australia.

Makita, A., J. Suzuki and Y. Suyama (2010) The bamboos – their mysterious life histories. Japanese Journal of Ecology 60 (1): 45-50.

Matsuo, A., Y. Suyama and A. Makita (2010) The relationship between rhizome habits and spatial distribution pattern of genets in two dwarf bamboos, *Sasa veitchii* var. *hirsuta* and *Sasa kurilensis*. Japanese Journal of Ecology, 60 (1): 81-88.

Nanzyo, M., H. Kanno and T. Takahashi (2010) Organic Carbon Accumulation in Andosols: (3) Occurrence of Apatite and Biotite in Young and Matured Volcanic Ash Soils, and Discussions on Nutrient Supply for Ecosystems. Journal of Integrated Field Science, 7: 73-77.

Nanzyo, M., H. Yaginuma, K. Sasaki, K. Ito, Y. Aikawa, H. Kanno and T. Takahashi (2010) Identification of vivianite formed on the root of paddy rice grown in pots. Soil Sci. Plant Nutr., 56: 376-381.

Nanzyo, M. (2010) Can we use phosphate abundantly accumulated in the cultivated Andosols? Pedologist, 54: 45-51.

Nanzyo, M. (2010) Chemical properties of volcanic ash soils-chemical models for their colloidal constituents and elemental concentrations, and plant availability of nutrient elements. Jpn. J. Soil Sci. Plant Nutr., 81:451-453.

Nanzyo, M. (2010) Phosphorus in soil. In: The behavior of phosphorus from the view point of socio cultural soil science, K. Minami and N. Mitsumata (Eds.), 129-146, Hakuyusha, Tokyo (Book).

Nanzyo, M., (2010) Role of mineral constituents in characterizing soils and nutrient supply for plants. Clay Sci., 14:247-251.

Oddou-Muratorio, S., A. Bontemps, E. K. Klein, I. Chybicki, G. G. Vendramin and Y. Suyama (2010) Comparison of direct and indirect genetic methods for estimating seed and pollen dispersal in *Fagus*

sylvatica and *Fagus crenata*. Forest Ecology and Management 259 (11): 2151-2159.

Saito, M. (2010) Significance of arbuscular mycorrhizal fungi in re-vegetation process in nitrogen-limited degraded ecosystems. J. Integrated Field Sciences, 7: 37- 40.

Seiwa, K. (2010) Scientific bases in the conversion of managed conifer forests to hardwood mixed forests. Forest Science, 59: 3-8 (in Japanese).

Seiwa, K. (2010) Is the Janzen–Connell hypothesis valid in temperate forests? Journal of Integrated Field Science, 7: 3-8.

Suyama, Y., J. Suzuki and A. Makita (2010) For the comprehension of gregarious flowering in bamboos. Japanese Journal of Ecology, 60 (1): 12-16.

Takahashi, T., K. Yamada, H. Kanno and M. Nanzyo (2010) Organic Carbon Accumulation in Andosols: (2) Contribution of Aluminum-humus Complexes to Carbon Accumulation in Non-allophanic Andosols. Journal of Integrated Field Science, 7: 69-72.

Takanishi, I., M. Hayatsu, K. Yagi and M. Saito (2010) Phosphate uptake by extraradical hyphae of AM fungi is regulated by mineral nitrogen concentration in soil, 9th International Mycological Congress, Edinburgh, UK.

Tateishi, M., T. Kumagai, Y. Suyama and T. Hiura (2010) Differences in transpiration characteristics of Japanese beech trees, *Fagus crenata*, in Japan. Tree Physiology, 30: 748-760.

Yao, Q., R. Ohtomo, R. and M. Saito (2010) Nitrogen and phosphorus influence on polyphosphate accumulation in *Gigaspora margarita* during spore germination. Plant and Soil, 330: 303-311.

The Ruminant Production Group

Akasaka, C., S. Ninomiya and S. Sato (2010) The effect of brushing by a human on behavioural and physiological stress indicators of cows. Abstract of the 8th International Symposium of Integrated Field Science, Sendai, Japan, pp.22.

Amer, S., R. Matsubara, F. Murakoshi and Y. Nakai (2010) Molecular analysis of *Cryptosporidium parvum* HNJ-1 isolated in Japan. J. Vet. Med. Sci., 72: 1647-1649.

Amer, S., F. Masoud, H. Honma, Y. Fukuda, C. Tada and Y. Nakai (2010) Multilocus genetic analysis of *Cryptosporidium parvum* form Egypt. Parasitol. Res., 107(5): 1043-1047.

Amer, S., H. Honma, M. Ikarashi, C. Tada, Y. Fukuda, Y. Suyama and Y. Nakai (2010) *Cryptosporidium* genotypes and subtypes in dairy calves in Egypt. Vet. Parasit., 169: 382-386.

Anjiki, A., T. Sato and S. Ninomiya (2010) The welfare assessment of stabled horses on the basis of behavioural observation throughout the year. The 44th International Congress of the International Society for Applied Ethology Abstracts, 191.

Asano, R., K. Otawa, Y. Ozutsumi, N. Yamamoto, A. Hosnia and Y. Nakai (2010) Development and analysis of microbial characteristics of an acidulocomposting system for the treatment of garbage and cattle manure. J. Biosci. Bioeng., 110(4): 419-25.

Asano, R., K. Kubori, Y. Ozutsumi, N. Yamamoto, K. Otawa and Y. Nakai (2010) Detection of Escherichia coli in a cattle manure composting process by selective cultivation and colony PCR. J. Environ. Sci. Health, Part B Pesticides, Food Contaminants, and Agricultural Wastes (Book, in press).

Fukuda, Y. and T. Suzaki (2010) A novel nuclear protein Np23 from the ancestral dinoflagellate *Oxyrrhis marina*. Jpn. J. Protozool., 43(1): 28-29 (in Japanease).

Fukuda, Y. and Y. Nakai (2010) The Dictionary of Biology (coauthor), H. Ishikawa et al. (Eds.), Tokyo-Kagaku Douzin (Book).

Hirata, M., S. Ogura and M. Furuse (2010) Fine-scale spatial distribution of herbage mass, herbage consumption and fecal deposition by cattle in a pasture under intensive rotational grazing. Ecological Research, Online 14 Dec.

Hirata, M. and S. Ogura (2010) Spatio-temporal variation of herbage mass in grazing pastures. In: Grassland Science Series 2: Ecology and Conservation of Grasslands – For Coordination of Livestock Production and Biodiversity. Ed. by the Japanese Society of Grassland Science, 260-271, Japan Scientific Societies Press, Tokyo (Book).

Hosnia, A, T. Sasaki, C. Tada and Y. Nakai (2010) Characterization and partial purification of a bacteriocin-like substance produced by thermophilic Bacillus licheniformis H1 isolated from cow-manure compost. Animal Sci. J. (in press).

Hosnia, A., N. Yamamoto, K. Otawa, C. Tada and Y. Nakai (2010) Isolation of bacteriocin-like substance producing bacteria from finished cattle-manure compost and activity evaluation against

some food-borne pathogenic and spoilage bacteria. J. Gen. Appl. Microbiol., 56 (2): 151-161.

Ikeda, N., C. Tada, S. Nakamura, J. Chigira, T. Yano, K. Nakano and Y. Nakai (2010) Removal of coliform bacteria in three types of constructed wetlands in a temperate area of Japan. Proceedings of 12th IWA International Conference on Wetland Systems for Water Pollution Control, vol. 2, 1332-1333.

Ito, A., H. Hnma, G. Gurelli, B. Gocmen, T. Mishima, Y. Nakai and S. Imai (2010) Redescription of Triplumaria selenica Latteur et al., 1970 (Ciliophora, Entodiniomorphida) and its phylogenetic position based on the infraciliary bands and 18SSU rRNA gene sequence. Europ. J. Protistology, 46: 180-188.

Kimura, K., T. Kobayashi, Y. Y. Li and Y. Nakai (2010) Analysis of bio-conversion and microbial community of thermophilic hydrogen-producting UASB process treating starch wastewater. Environmental Engineering Research, 47: 349-356 (in Japanese).

Kitagawa, A., C. Akasaka, S. Ninomiya and S. Sato (2010) The validity of salivary-amylase as stress marker in dairy cattle. Abstract of the 8th International Symposium of Integrated Field Science, Sendai, Japan, p. 23.

Koda, H., C. Oyakawa and A. Lemasson (2010) Mother-offspring overlapping duetting in gibbons. Primate Research, p.39. Mother-offspring overlapping duetting in gibbons. Proceedings of the International Primatological Society XXIII Congress, p.39.

Lemasson, A., H. Koda, A. Kato, C. Oyakawa, C. Blois-Heulin and N. Masataka (2010) Influence of sound specificity and familiarity on Japanese macaques' (Macaca fuscata) auditory laterality. Behavioural Brain Research, 208(1): 286-289.

Nakai, Y. (2010) Japanese legal system of murrain. The Toshi Mondai, 101 (10): 46-54 (in Japanese).

Ninomiya, S. and S. Sato (2010) The assessment of the effect of presenting a companion's face picture on social isolation stress using saliva sampling in cows. Animal Science Journal (in press).

Ninomiya, S. (2010) Welfare Assessment in Horses (Benessere negli equine). In: Benessere Animale (Animal Welfare). Fulvio Biancifiori (Ed.), Litograf Editor srl, Citta di Castello (Pg), 215- 244 (Book, in Italian).

Ogura, S., Y. Iino and S. Sato (2010) Effects of vegetational characteristics and foraging behavior at a feeding station scale on home range size in grazing cattle. Tohoku Journal of Animal Science and Technology, 59(3): 41-48 (in Japanese with English summary).

Ogura, S. (2010) Diet selection and foraging behavior of cattle in species-rich vegetation. Abstract of the 8th International Symposium of Integrated Field Science, Sendai, Japan, p. 11.

Ogura, S., T. Shishido, S. Tanaka, K. Yusa and S. Sato (2010) Effect of application of cattle compost to temperate meadow on herbage productivity and quality. Annual Report of the Project of Integrated Compost Science (PICS), 44-51.

Ogura, S. (2010) Forage value of Japanese native plants for grazing livestock. – Importance of forage evaluation focusing on the function of rumen. In: Grassland Science Series 2: Ecology and Conservation of Grasslands – For Coordination of Livestock Production and Biodiversity. Ed. by the Japanese Society of Grassland Science, Japan Scientific Societies Press, Tokyo, 139-155.

Ohara, A., M. Tanimura and S. Sato, S. (2010) The effect of environmental enrichment on welfare and productivity in homebred broiler strain "Tatsuno". Abstract of the 8th International Symposium of Integrated Field Science, Sendai, Japan, p. 25.

Ohara, A (2010) Response of broiler company to animal welfare in Europe. Sustainable Livestock Production and Human Welfare, 64(6): 613-617 (in Japanese).

Okada, M., Y. Yoshihara and S. Sato (2010) Effect of disturbance size on similarity and resilience of vegetation to the disturbance. Abstract of the 8th International Symposium of Integrated Field Science, Sendai, Japan, p. 30.

Oyakawa, C., K. Sato and S. Sato (2010) Is the dairy cow performing to bite grasses comfortable in grazing? Abstract of the 8th International Symposium of Integrated Field Science, Sendai, Japan, p. 26.

Oyakawa, C., H. Koda, T. Tanaka, T. Murai, S. Nulukamilah, A. Rizaldi Bakar and N. Masataka (2010) Geographic variation of species-specific call in wild agile gibbons. Proceedings of the International Primatological Society XXIII Congress, p. 49.

Sasaki, Y., Y. Yoshihara, J. Undarmaa and T. Ohkuro (2010) Ecological stoichiometry explains larger-

scale facilitation processes by shrubs on species coexistence among understory plants. Ecological Engineering, 36: 1070-1075.

Sato, S. (2010) The Japanese guideline for animal welfare in livestock production (1) Why was it made? Where does the animal husbandry system go? CHIKUSANKAIKEIEIJYOUHOU, 243: 11-14 (in Japanese).

Sato, S. (2010) The Japanese guideline for animal welfare in livestock production (2) Animal welfare improves animal productivity. CHIKUSANKAI-KEIEIJYOUHOU, 244: 1-4 (in Japanese).

Sato, S. (2010) Animal welfare in livestock production- with the focus on the animal welfare standard in the Terrestrial Code of OIE. Miyagi Veterinarian, 63(2): 48-54 (in Japanese).

Sato, S., T Tanaka, M Fukasawa (2010) EU goes for animal welfare in livestock production- Final conference on EU funded project Welfare Quality. Sustainable Livestock Production and Human Welfare, 64(6): 599-612 (in Japanese).

Sato, S. (2010) Verifying deliciousness of pork from grazed pigs. GENDAINOUGYOU, 765: 252-255. (in Japanese).

Sato, S. (2010) Some effecting factors and improving factors on the stress of dairy cows- with reference to assessment protocol for animal welfare-. Dairy Journal, 63(8): 12-15 (in Japanese).

Sato, S. (2010) Efforts on animal welfare in the world 1. Principles and movements on animal welfare. Poultry Research, special number 7: 5-7 (in Japanese).

Sato, S. (2010) Current situation of animal welfare at home and abroad and the relationship between animal welfare and animal production. KAGAKUSH-IRYOU, 55(9): 337-343 (in Japanese).

Sato, S. (2010) Complexity is irresistible attractive – Animal behaviour science, animal welfare, and technical development of animal husbandry. SEEDer, 3: 66-69 (in Japanese).

Shinomiya, T., K. Sato, S. Tanaka, S. Ninomiya and S. Sato (2010) Comparing welfare of dairy cows in the grazing system and the housing system. The Proceedings of The 14th AAAP Animal Science Congress, 1249-1252.

Tanaka, M., Y. Kamiya, T. Suzuki and Y. Nakai (2010) Effect of citrus pulp silage feeding on concentration of beta-cryptoxanthin in plasma and milk of dairy cows. Anim. Sci. J., 81(5): 569-573.

Tomimatsu, H., M. Yokoyama and S. Ogura (2010) Quantification of bite size, bite rate and intake rate in cattle foraging Japanese native grasses. Abstract of the 8th International Symposium of Integrated Field Science, Sendai, Japan, p. 31.

Uetake, K, T. Tanaka and S. Sato (2010) Effects of haul distance and stocking density on young suckling calves transported in Japan. Animal Science Journal (in press).

Yamamoto, N., K. Otawa and Y. Nakai (2010) Diversity and Abundance of Ammonia-Oxidizing Bacteria and Ammonia-Oxidizing Archaea During Cattle Manure Composting. Microbial Ecology, 60 (4): 807-815.

Yamamoto, N., M. Tannai and Y. Nakai (2010) Research on waste biomass recycling: the two-year trial for composting of residues of fruits and vegetables. Bulletin of Integrated Field Science Center, (accepted) (in Japanese).

Yamamoto, Y, Y. Deguchi, H. Tsukada, S. Sato, M. Kitagawa, T. Nishida, H. Terada, K. Matoba, K. Ikeda, M. Sato and K. Hirano (2010) Vegetation, Dry Matter Production and Consumption of Established Pasture for Gathering Cattle in a Vast Forest Grazing Land. Japanese Journal of Grassland Science, 56: 137-143 (in Japanese).

Yokoyama, M., M. Hori and S. Ogura (2010) Do cattle choose preferred plant species without visual sense? Abstract of the 8th International Symposium of Integrated Field Science, Sendai, Japan, p. 32.

Yokota, S., N. Ikeda, Y. Suzuki, S. Furusawa, S. Ogura and S. Sato (2010) Consumer's trend of beef, pork and chicken in Japan. Bulletin of Integrated Field Science Center, 26: (in press, in Japanese)

Yoshihara, Y., T. Ohkuro, B. Buuveibaatar, J. Undarmaa and K. Takeuchi (2010) Spatial pattern of grazing affects influence of herbivores on spatial heterogeneity of plants and soils. Oecologia, 162: 427-434.

Yoshihara, Y., T. Ohkuro, B. Buuveibaatar, J. Undarmaa and K. Takeuchi (2010) Clustered animal burrows yield higher spatial heterogeneity. Plant Ecology, 206: 211-224.

Yoshihara, Y., T. Ohkuro, B. Buuveibaatar, J. Undarmaa and K. Takeuchi, K. (2010) Responses of vegetation to soil disturbance by Sibelian marmots within a landscape and between landscape positions in Hustai National Park, Mongolia. Grassland

Science, 56: 42-50.
Yoshihara, Y., T. Ohkuro, B. Buuveibaatar, J. Undarmaa and K. Takeuchi (2010) Complementary effects of disturbance by livestock and marmots on the spatial heterogeneity of vegetation and soil in a Mongolian steppe ecosystem. Agriculture Ecosystems and Environment, 135: 155-159.
Yoshihara, Y., T. Ohkuro, B. Buuveibaatar, J. Undarmaa and K. Takeuchi (2010) Pollinators are attracted to mounds created by burrowing animals (marmots) at a Mongolian grassland. Journal of Arid Environments, 74: 159-163.
Yoshihara, T., T. Sasaki, T. Okuro, J. Undarmaa and K. Takeuchi (2010) Cross-spatial-scale patterns in the facilitative effect of shrubs and potential for restoration of desert steppe. Ecological Engineering, 36: 1719-1724.
Yoshihara, Y. (2010) Predicting plant species diversity and changes in productivity due to simulated effects of cattle disturbances in Japanese grasslands. Abstract of the 8th International Symposium of Integrated Field Science, Sendai, Japan, p. 13.
Yoshihara, Y. (2010) Integrating ecosystem engineering and spatial heterogeneity concepts: toward biologically diverse Mongolian steppe. Global Environmental Research, 14: 55-62.

The Rice Production Group

Irving, L. J., Y. Suzuki, H. Ishida and A. Makino (2010) Protein turnover in grass leaves. Advances in Botanical Research, 54: 139-182.
Ishida, H, Y. Suzuki and A. Makino (2010) Rubisco turnover and photosynthesis in a leaf. In: Nitrogen Assimilation in Plants: Chapter 17, T. Ohyama and K. Sueyoshi (Eds.), 277-285, Research Signpost, Kerala.
Ito, T. (2010) Possibility of practical use of water purification sludge with polysilicate-iron flocculant in environmentally sound rice production. J. Water and Waste, 52: 76-82 (in Japanese).
Ito, T. (2010) Effects of paddy-upland rotation on soil phosphorus availability. In Fertility and manegement of soil in paddy-upland rotation fields in Japan. Ed. by Japanese Society of Soil Science and Plant Nutrition, Hakuyusha, Tokyo, 122-136 (Book, in Japanese).
Ito, T. and K. Hara (2010) Impact of tubificid worm on nutrient dynamics in paddy field. J. Integ. Field Sci., 7: 47-50.
Ito, T., K. Hara, M. Kawase and C. Kon (2010) Effects of tubificid worms on soil properties in rice fields with organic farming. 19th World Congress of Soil Science, Brisbane, Australia.
Izumi, M, S. Wada, A. Makino and H. Ishida (2010) The autophagic degradation of chloroplasts via Rubisco-containing bodies is specially linked to leaf carbon status but not nitrogen status in Arabidopsis. Plant Physiol., 154: 1196-1209.
Kanno, K and A. Makino (2010) Increased grain yield and biomass allocation in rice under cool night temperature. Soil. Sci. Plant Nutr., 56: 412-417.
Kato, Y. M. Okami, R. Tajima, D. Fujita and N. Kobayashi (2010) Root response to aerobic conditions in rice, estimated by Comair root length scanner and scanner-based image analysis. Field Crops Research, 118: 194-198.
Mae, T. and A. Makino (2010) Nitrogen utilization, growth and yield in rice plants. In: Nitrogen Assimilation in Plants, Chapter 17, T. Ohyama and K. Sueyoshi (Eds.), 243-253, Research Signpost, Kerala.
Nakano, R, H. Ishida, M. Kobayashi, A. Makino and T. Mae (2010) Biochemical aspects associated with in vivo RbcL fragmentation by reactive oxygen species under chilling-light conditions. Plant Biology, 12: 35-45.
Sano, O., T. Ito, T. Ando, M. Nanzyo, G. Saito and M. Saigusa (2010) Clay mineralogical composition of representative paddy soils in southern part of northeastern Japan. Pedologist, 54: 83-92 (in Japanese).
Suzuki, Y, T. Kihara-Doi, T. Kawazu, C. Miyake and A. Makino (2010) Differences in Rubisco content and its synthesis and degradation in leaves at different positions in Eucalyptus globulus seedlings. Plant Cell Environ., 33: 1314-1323.

Marine Bio-Production Group

Ciocan, C. M., E. Cubero-Leon, A. M. Puinean, E. M. Hill, C. Minier, M. Osada, K. Fenlon and J. M. Rotchell (2010) Effects of estrogen exposure in mussels, *Mytilus edulis*, at different stages of gametogenesis, Environmental Pollution, 158: 2977-2984.
Ciocan, C. M., M. A. Puinean, E. Cubero-Leon, E. M. Hill, C. Minier, M. Osada, N. Itoh and J. M.

Rotchell (2010) Endocrine Disruption, Reproductive Cycle and Pollutants in Blue Mussel Mytilus edulis. In: Interdisciplinary Studies on Environmental Chemistry Vol. 3 -Biological Responses to Contaminants: From Molecular to Community Level. N. Hamamura, S. Suzuki, S. Mendo, C. M. Barroso, H. Iwata and S. Tanabe (Eds.), TERRAPUB, Tokyo, 121-126.

Cubero-Leon, E., C. M. Ciocan, E. M. Hill, M. Osada, M. Kishida, N. Itoh, R. Kondo, C. Minier and J. M. Rotchell (2010) Estrogens disrupt *serotonin receptor* and *cyclooxygenase* mRNA expression in the gonads of mussels (*Mytilus edulis*), Aquat. Toxicol., 98: 178-187.

Hirase, S., M. Kanno and A. Kijima (2010) Isolation and characterization of 15 microsatellite DNA loci for assessing population structure of the intertidal goby, *Chaenogobius annularis* (Gobiidae). Molecular Ecology Resources, 10: 1106-1108.

Kobayashi, T. and A. Kijima (2010) Effects of inbreeding depression in Pacific abalone *Haliotis discus hannai*. Journal of Shellfish Research, 29: 643-649.

Ikeda, M., W. Endo, T. Fujii, M. Ishii, Y. Akaba, M. Nakamura, T. Isshiki and S. Katayama (2010) Genetic uniqueness and demographic histories of marbled sole populations in Tokyo and Ise-Mikawa Bays. Fisheries and Environments in Tokyo Bay, 1: 7.

Itoh, N, R. Kamitaka, K. G. Takahashi, M. Osada (2010) Identification and characterization of multiple <beta>-glucan binding proteins in the Pacific oyster, *Crassostrea gigas*. Dev. Comp. Immunol., 34: 445-454.

Itoh, N., Y. Okada, K. G. Takahashi., M. Osada (2010) Presence and characterization of multiple mantle lysozymes in the Pacific oyster, *Crassostrea gigas*. Fish and Shellfish Immunol., 29: 126-135.

Romana-Eguia, M. M. R., M. Ikeda, Z. U. Basiao and N. Taniguchi (2010) Growth comparison of Asian Nile and red tilapia strains in controlled and uncontrolled farm conditions. Aquaculture International, 18: 1205-1221.

Tanabe, T., Y. Yuan, S. Nakamura, N. Itoh, K. G. Takahashi and M. Osada (2010) The role in spawning of a putative serotonin receptor isolated from the germ and ciliary cells of the gonoduct in the gonad of the Japanese scallop, *Patinopecten yessoensis*. Gen. Comp. Endocrinol., 166: 620-627.

Tanimoto, S., N. Itoh, M. Osada and K. G. Takahashi (2010) Agglutination of bacteria by a lectin in the hemolymph of the Pacific oyster, Crassostrea gigas. Tohoku J. Agr. Res., 60: 93-90, 2010.

Vargas, K., K. Hamasaki, Y. Asakura, Y., K. Nakajima, M. Ikeda, K. Tsuchiya, N. Taniguchi and S. Kitada (2010) Genetic diversity and differentiation in allozymes and shell sculpture of the clams *Ruditapes spp*. in natural populations of Japan and China. Fish Genetics and Breeding Science, 40: 37-46.

Xu, K., M. Kanno, H. Yu, Q. Li and A. Kijima (2010) Complete mitochondrial DNA sequence and phylogenetic analysis of Zhikong scallop *Chlamys farreri* (Bivalvia: Pectinidae). Molecular Biology Report, Online first.

Integrated Field Control Group

Hiraguchi, Y., S. Nishihashi and K. Morozumi (2010) The Function of rural community and their contributions for sustainable development in mountainous area: A case study of O-Village in Rikuzentakata City, Iwate Prefecture. Journal of Farm Management Economics, 41: 49-68 (in Japanese).

Hori, M., C. Yonezawa, D. Kunii, Y. Yoshihara and H. Suzuki (2010) Estimation of Grassland Condition in Mongolia from Field Survey and Satellite Images. Proceedigs of the 31st Asian Conference on Remote Sensing, CD-ROM.

Imai, T., D. Kunii and C. Yonezawa (2010) Full Polarimetric Observation of Rice Growth by PALSAR, Proceedigs of the 31st Asian Conference on Remote Sensing, CD-ROM.

Kitamura, A., D. Kunii, C. Yonezawa and G. Saito (2010) Monitoring of Paddy Rice Fields in Australia Using ASTER Data and TM Data. Proceedigs of the 31st Asian Conference on Remote Sensing, CD-ROM.

Morozumi, K. (2010) Establishment of Cycle-Type River Basin Economic Zone and Creation of Regional Environmental business –A Case of Charcoal Electric Power-, Hito to Kokudo21 (Human and Land21), 36(1): 26-29 (in Japanese).

Morozumi, K. (2010) An importance of rice-ethanol fuel and problems that Japanese Government confronts- From a view point of “Rice is a regional resource”. In: Production of bio-fuel with rice and vitalization of rural societies-Problems of energy,

food and environment-, M. Yabe and K. Morozumi (Eds.), 1-283, Tsukubashobo, Tokyo, 15-39 (Book, in Japanese).

Morozumi, K. (2010) Conclusions and some issues that we discuss in future. In: Production of bio-fuel with rice and vitalization of rural societies-Problems of energy, food and environment-, M. Yabe and K. Morozumi (Eds.), 1-283, Tsukubashobo, Tokyo, 273-281 (Book, in Japanese).

Morozumi, K. (Ed.) (2010) Integrated regional management in the cycle-type river basin economic zone. The Creation of an entity that can promote a regional environmental business and social system-interim report-, Report for Grants-in-Aid for Scientific Research(Grant-in-Aid for Scientific Research(B)-, K. Morozumi, 1-89 (in Japanese).

Morozumi, K. (Ed.) (2010) For the realization of "Oisawa Ecomusee"-Interim Report- (Research and Development Project: "Community-Based Actions against Global Warming and Environmental Degradation", Research Institute of Science and Technology for Society, 2008.10-2010.3, Project Director, K. Morozumi, 1-74 (in Japanese).

Morozumi, K. (Ed.) (2010) Community-based decentralized energy societies in Tohoku district-Final Report- (Research and Development Project :" Community-Based Actions against Global Warming and Environmental Degradation", Research Institute of Science and Technology for Society, 2008.10-2010.3, Project Director, K. Morozumi, 1-84

Osawa, K., D. Kunii and G. Saito (2010) Classification of Arable Land at Osaki Area in Miyagi Prefecture Using Multi-Temporal Terra/ASTER Data. The Journal of Japanese Agricultural Systems Society, 26(2): 67-77 (in Japanese with English abstract).

Saito, G., C. Yonezawa, A. Kitamura, S, Ogawa and M. Kato (2010) Determination of Agricultural Characteristics in Each Country Using TERRA/ASTER Data. Proceedings of ISPRS Technical Commision VIII Symposium.

Saito, M. (2010) Non-CO_2 Greenhouse Gases (GHG) Emission from Natural Processes in Carbon Footprint of Foods, 9th Conference of EcoBalance, Tokyo, Tokyo Mirai-Kan.

Sato, N., C. Yonezawa, D. Kunii, M. Hori and G. Saito (2010) Temporal Change Observation on Damage Situation of the Iwate-Miyagi Nairiku Earthquake in 2008 Using Alos Satellite Images. Proceedigs of the 31st Asian Conference on Remote Sensing, CD-ROM.

Watanabe, H., D. Kunii and C. Yonezawa (2010) Observation of Tsunami Damage to Marine Farm on Coast in Tohoku District Caused by the 2010 Chili Earthquake Using ALOS Satellite. Proceedigs of the 31st Asian Conference on Remote Sensing, CD-ROM.

Yabe, K., D. Kunii, C. Yonezawa, S. Ogawa, Y. Kosugi and G. Saito (2010) Classification of Coniferous Tree Species Using Aerial Hyper Spectral Observation 2, Proceedigs of the 31st Asian Conference on Remote Sensing, CD-ROM.

Yonezawa, C., G. Saito, M. Watanabe, N. Ishitsuka, M. Negishi, H. Muraki, K. Azuma and S. Ogawa (2010) Paddy field observation by single- and full-polarimetric RADARSAT-2 data. Proceedings of the 48th spring conference of the remote sensing society of Japan, 74-75 (in Japanese with English abstract).

Yonezawa, C., G. Saito, M. Hori, M, Watanabe (2010) Continuous Monitoring of Landslides Area Caused by Iwate-Miyagi Nairiku Earthquake in 2008 Using ALOS Images. Proceedings of ISPRS Technical Commision VIII Symposium.

8th International Symposium of Integrated Field Science

Advanced Studies on Sustainable Animal Production: Interrelationships among Human, Animal and Environment

Date: 18 (Sat.) – 19 (Sun.) September, 2010
Venue: Multimedia Hall, Kawauchi-Kita Campus,
Tohoku University

Co-organized Japanese Society of Grassland Science
The Animal Production Environment Society of Japan
Japanese Society for Applied Animal Behaviour

Symposium Chair
Prof. Shusuke SATO (Integrated Field Science Center, Tohoku University, Japan)
Prof. Yutaka NAKAI (Project of Integrated Compost Science, Tohoku University, Japan)

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---------- Program ----------

18 (Sat.) September

09:00–09:05 Opening Address: Masanori SAITO (Tohoku University, Japan)

09:05–09:10 Scope of the Workshop: Yutaka NAKAI (Tohoku University, Japan)

09:10–12:15 Session 1. Improving Animal Welfare: Multidimentional Approach

1-1. Environmental Enrichment and Human Contact with Animal
Shigeru NINOMIYA (Tohoku University, Japan) 09:10–09:40

1-2. The Investigation of Slaughter House about the Welfare of Pig and Broiler in Korea
Hwang LEE (National Veterinary Research & Quarantine Service, Korea) 09:40–10:10

1-3. The On-Farm Assessment of Animal Welfare in Dairy Cattle
Tetsuya SEO (Obihiro University, Japan) 10:10–10:40

----- Tea Break ----- 10:40–10:55

1-4. Genes, Neurons, and Circuits Underlying the Reproductive Behavior in *Drosophila*
Kosei SATO (Tohoku University, Japan) 10:55–11:25

1-5. The Role of Oxytocin in Animal Behaviour and Welfare
Katsuhiko NISHIMORI (Tohoku University, Japan) 11:25–11:55

Discussion
Chairperson: Shigeru NINOMIYA (Tohoku University, Japan) 11:55–12:15

12:15–13:15 Lunch

13:15–14:15 Poster Viewing

14:15–17:20 Session 2. Contribution of Grazing on Food Production and Ecological Conservation of Grassland Ecosystems

2-1. Diet Selection and Foraging Behavior of Cattle in Species-rich Vegetation
Shin-ichiro OGURA (Tohoku University, Japan) 14:15–14:45

2-2. Conservation Management of Livestock Grazing and Wildlife in Mongolian Grassland
Buyanaa CHIMEDDORJ (WWF Mongolia, Mongolia) 14:45–15:15

2-3. Predicting Plant Species Diversity and Changes in Productivity Due to Simulated Effects of Cattle Disturbances in Japanese Grasslands
Yu YOSHIHARA (Tohoku University, Japan) 15:15–15:45

----- Tea Break ----- 15:45–16:00

2-4. Effect of Grazing on Habitat and Behavior of Wild Mammals
Hideharu TSUKADA (National Institute of Livestock and Grassland Science, Japan) 16:00–16:30

2-5. Forage Production, Utilization and Environmental Conservation in Sweden
Magnus HALLING (Swedish University of Agricultural Science*, Sweden) 16:30–17:00
**Academic Exchange Cooperation between the University and Graduate School of Agricultural Science, Tohoku University*

Discussion
Chairperson: Shin-ichiro OGURA (Tohoku University) 17:00–17:20

19:00–21:00 Banquet (Hotel JAL City Sendai)

19 (Sun.) September

9:00–12:05 Session 3. Advanced Technology on Recycling of Animal Wastes

3-1. Animal Wastewater Treatment Using Constructed Wetland
Chika TADA (Tohoku Univeristy, Japan) 09:00–09:30

3-2. Introduction of Biomass Accounting as an Evaluation Tool of Biomass Utilization Systems: a Case Study of Domestic Animal Waste Treatment Oriented Biomass Town
Lyudmyla BESPYATKO (National Institute of Advanced Industrial Science and Technology, Ukraine) 09:30–10:00

3-3. Power Generation from Animal Wastewater Using a Single-chamber Microbial Fuel Cell
Osamu ICHIHASHI (Oak Ridge National Laboratory, USA) 10:00–10:30

----- Tea Break ----- 10:30–10:45

3-4. Archaeal Community During Cattle Manure Composting Process in Field-scale Facility
Nozomi YAMAMOTO (Tohoku University, Japan) 10:45–11:15

3-5. Development of a New Fermentation Process for Hardly Degradable Rice Straw Agriculture Waste
Yingnan YANG (University of Tsukuba, Japan) 11:15–11:45

Discussion
Chairperson: Chika TADA (Tohoku University, Japan) 11:45–12:05

12:05–12:10 Closing Remarks: Shusuke SATO (Tohoku University, Japan)

12:10– Field Tour

Poster Session

1	C. Akasaka et al.	Tohoku University	The effect of brushing by a human on behavioral and physiological stress indicators of cows
2	A. Kitagawa et al.	Tohoku University	The validity of salivary-amylase as stress marker of cattle
3	A. Morimoto et al.	Shinshu University	Overall animal welfare assessment of dairy cattle at the farm level using the Shinshu comfort livestock farm certification standard
4	A. Ohara et al.	Tohoku University	The effect of environmental enrichment on welfare and productivity in homebred strain “Tatsuno”(chicken for meat)
5	C. Oyakawa et al.	Tohoku University	Is the dairy cow performing to bite grasses comfortable in grazing?
6	K. Takeda et al.	Shinshu University	Does housing conditions affect the flight distance of dairy cows?
7	K. Kakinuma et al.	University of Tokyo	The linkage between pastoralists’ perspectives and vegetation threshold changes in mongolian rangelands
8	A. Yamada et al.	National Agricultural Research Center for Kyushu Okinawa region	Recovery of *pleioblastus variegatus* from flowering in the Aso region-results of the 17th year-
9	M. Okada et al.	Tohoku University	Effect of disturbance size on similarity and resilience of vegetation to the disturbance
10	H. Tomimatsu et al.	Tohoku University	Quantification of bite size, bite rate and intake rate in cattle foraging Japanese native grasses
11	M. Yokoyama et al.	Tohoku University	Do cattle choose preferred plant species without visual sense?
12	K. Kimura et al.	Tohoku University	Analysis of bioconversion and bacterial community of thermophilic hydrogen production using uasb reactor
13	Y. Baba et al.	Tohoku University	Methane fermentation from waste glycerol and sludge ~approach on Miyagi prefecture 3 R new technical research development project~
14	R.Watanabe et al.	Tohoku University	Prevalence and distribution of bovine coccidia in the northern area of Miyagi prefecture
15	R. Sato et al.	Tohoku University	Methane fermentation using waste glycerol produced from biodiesel fuel
16	E. Ito et al.	Kanaashi Agricultural Senior High School	Cattle is possible host animal of the land leech
17	D. Kunii et al.	Tohoku University	Availability of forest resources as firewood for preservation of regional environment

Oral Session
(Invited Speakers)

1-1. Environmental Enrichment and Human Contact with Animal

Shigeru NINOMIYA

Tohoku University, Japan

In animal welfare, we focus on the physical and mental health of the animals with reference to “Five freedoms.” Five freedoms that farm animals are entitled to are as follows: (1.) freedom from hunger and thirst; (2.) freedom from discomfort; (3.) freedom from pain, injury, or disease; (4.) freedom to express normal behavior; and (5.) freedom from fear and distress (FAWC 1992). We also consider animal welfare on some phases of livestock production system, such as management and environment in farm, transport and slaughterhouse. In recent years, the animal welfare standard for transport, slaughter and killing has been established and on-farm animal welfare assessment has developed. The methods of rearing livestock have been studied for animal welfare such as in nutritional studies about feed that satisfies their nutritive requirement (freedom from hunger), studies about environmental resources such as bedding and ventilation (freedom from discomfort), and veterinary studies about preventing injury or disease (freedom from injury or disease). It is also important to investigate the psychological aspects of animals in terms of freedom to express normal behavior and freedom from fear and distress. Environmental enrichment and establishing human-animal bond (for example, brushing) as the methods for improving the psychological aspects of animals in intensive animal husbandry is known. Environmental enrichment is not only for livestock animals and in laboratory animals such as rat and fruit flies. It was reported that environmental enrichment could improve biological function of laboratory animals.

1-2. The Investigation of Slaughter House about the Welfare of Pig and Broiler in Korea

Hwang LEE

Ministry for Food, Agriculture, Forestry and Fisheries, Korea

The importance of animal welfare becomes conspicuous and now it is one of big global issues. The OIE established the animal welfare standard for transport, slaughter and killing and nowadays put their effort to make the standard for livestock production system. To cope with the global animal welfare trend, Korean government persevere efforts but the lack of field investigation and scientific background is one of difficult point. This investigation was carried out to know the actuality of animal welfare in slaughterhouse. The 23 pig slaughterhouses and 11 broiler slaughterhouses that were chosen by size and location and the vehicles that were used to transport pigs and broilers were investigated. The average distance from farm and slaughterhouse is 71.2km(pig) and 106.5km(broiler), and stocking density is 0.37 m^2/head(pig) and 167cm^2/ kg(broiler), respectively. Most pig slaughterhouse used electric goads and prods at the race way to the electric stunner. The proportion of pigs that was estimated as 'regained consciousness' after stunning is 12.3%(electric stunning) and 1.7%(CO_2 stunning). The electric voltage, current and time duration for stunning of broilers are 40~70V, 80~200mA, 6.6~10 sec. respectively. The average percentage of broilers that was estimated as regained consciousness is 8.9%(1.1%~16.5%). These results are not good when compared with animal welfare leader countries but it is meaningful because it is the first synthetic investigation of animal welfare in slaughterhouse of Korea.

1-3. The On-farm Assessment of Animal Welfare in Dairy Cattle

Tetsuya SEO

Obihiro University of Agriculture & Veterinary Medicine, Japan

Although concern related to farm animal welfare has increased worldwide, that expressed by Japanese consumers and producers remains moderate. Nevertheless, scientific research is necessary to prepare for the rising future concerns that are expected. We developed a system that supports overall on-farm assessment of dairy cow welfare based on published scientific reports.The assessment system includes 54 items reflecting resource-based, management-based and animal-based measures. All are designed to be as objective as possible. Each is assessed according to a two-point scale according to whether or not a criterion is met. We assessed 44 dairy herds kept in the free-stalls or tie-stalls on Japanese dairy farms. Results, which varied considerably among herds, show that the ranges of scores were 33-77% in resource-based, 41-82% in management-based, and 60-94% in animal-based items. Among all items, those for which 80% or more herds did not meet the criterion were crossing passages in free-stalls, calving pens, bed softness, feeding mangers, and hock condition. However, this assessment system remains incomplete; it must be refined, especially in terms of veterinary issues. Nations must establish their own animal welfare assessment systems in the future to address expected external pressures from other countries. Educational dairy farms are important so that consumers can learn directly from producers and experience actual animal farming including animal welfare. Moreover, is important to build animal welfare certification systems that consumers can support by purchasing animal-welfare-compatible products.

1-4. Genes, Neurons, and Circuits Underlying the Reproductive Behavior in *Drosophila*

Kosei SATO, M. KOGANEZAWA, G. TOBA and D. YAMAMOTO

Tohoku University, Japan

Instinct is organized by genetic programs that preconfigure the appropriate neural circuitry. Genetic dissection in model organisms thus has the potential to reveal the genes, neurons, and circuits underlying such behaviors, allowing us to determine the principle for instinct. With this goal in mind, we are studying the male courtship ritual of *Drosophila melanogaster*. This instinctive behavior is specified by the male-specific products of the *fruitless* (*fru*) gene, Fru, which are expressed in ~ 2000 cells in the male central nervous system. The *fru* mutant males show enhanced intermale courtship, with a concomitant decline in the activity to court to and copulate with females. The enhanced homosexual courtship in *fru* mutant males is considered to result from de-masculinization of a part of the brain due to loss-of-function of the *fru* gene. In fact, some *fru*-expressing neurons are sexually dimorphic in their numbers and projections. These neurons are completely feminized in males who are loss-of-function *fru* mutants. To understand molecular and cellular bases of Fru functions, we performed genetic screening for modifiers of *fru*, with the aim at determining their cofactors and targets. We show evidence that the *longitudinal lacking* (*lola*) gene genetically interacts with *fru* to generate neural sexual dimorphism and therefore contributes to gender specificity of behavior. This gene produces an enormous numbers of different BTB-Zn finger transcription factors through alternative splicing of its primary mRNA, and likely to function as a major regulator of axon patterning by controlling expression of multiple guidance molecules such as Slit and Robo.

1-5. The Role of Oxytocin in Animal Behaviors and Welfare

Katsuhiko NISHIMORI

Tohoku University, Japan

Neurohypophysial hormone oxytocin (OXT) was known to have an essential function in parturition and milk ejection. Recent studies including ours have also shown that OXT in a brain acts as an important neuromodulator to regulate several social behaviors, such as maternal behavior, mother/infant relationship, social recognition, male aggression and so on. We generated model disease mice lacking *Oxt* or *Oxtr* gene, and with them, we've continuously studied the physiological functions of OXT/OXTR in a way of behavioral neuroscience. We further generated the Oxtr-Venus knockin mice[1] to elucidate the localization of OXTR in brain, and found that OXTR was expressed in many nuclei in a brain, which were related to the regions controlling social behaviors. Many research groups reported the importance of OXT system to establish social relationship between individual animals, including model experimental animal, dog, and even human. Moreover, recent reports demonstrated that the intranasal administration of OXT improved emotion recognition of ASD (autistic spectrum disorders) patients. These results also imply the importance of OXT system from the viewpoint of animal welfare, because the concentration of OXT or distribution and expression level of OXTR may reflect the stress level of animals. Moreover, molecular-genetic breeding with modification of *Oxt* and /or *Oxtr* genes may potentially generate new domestic creatures with more stabilized mind and resistance to higher stress.

1) Yoshida, M., et al., *J.Neuroscience* **29**;2259 (2009)

2-1. Diet Selection and Foraging Behavior of Cattle in Species-rich Vegetation

Shin-ichiro OGURA

Tohoku University, Japan

The mechanism of diet selection and foraging behavior of large herbivores has given much concern to researchers because it often gives profound effects on the productivity and healthiness of the animals, and vegetational change. Particularly in extensive grazing systems, understandings of this plant-animal interaction are vital for adequate control of vegetation and animal conditions, and sustainable use of the plant resource. In this paper, factors affecting diet selection and foraging behavior of large herbivores were discussed in terms of accessibility, forage quality and other chemical factors. Previous studies showed that Japanese native pastures composed of 61-155 plant species, of which cattle graze upon 26-76. Among these species, Japanese plume-grass (*Miscanthus sinensis*) was most frequently grazed by cattle. Both horizontal and vertical distribution of available forage was a major factor affecting selectivity and ingestive behavior of cattle. Controlled experiments also proved that forage selectivity was affected by its height and animals selected forage more strongly from the height with higher intake rate. Estimated bite size of major native plants collected by hand-clipping well explained relative preference among plant species. In contrast, concentration of nutrients (digestible energy, crude protein and minerals) of the hand-clipping sample did not explain the relative preference of the plants. This is partly because toxic plants, *Hydrangea macrophylla* and *Pteridium aquilinum*, had high nutrient concentration. In addition, these toxic plants released specific volatiles. These findings warrant a further study for the effects of plant chemicals stimulating to scent and taste on diet selection of large herbivores.

2-2. Conservation Management of Livestock Grazing and Wildlife in Mongolian Grassland

Buyanaa CHIMEDDORJ

WWF Mongolia Programme Office, Mongolia

Mongolia's vast grasslands constitute approximately 70 percent of the country's 1.5 million square kilometers and fall into three major ecological zones: mountain-steppe, steppe, and desert-steppe. Mongolia's grasslands, including those in forested areas, high mountain pastures and true desert, these rangelands are the basis of livestock production and support over 40 million head of livestock (camels, cattle, yaks, horses, sheep and goats). The Mongolian part of Altai Sayan Ecoregion is a home of a diverse habitat mosaic of grazing pastures that sustain a number of globally endangered species. The southern part of the Mongolian Altai Sayan Ecoregion is characterized by flat plains, low mountains, rolling hills, and hillocks covered by different types of steppe and a network of lakes and wetlands. This area is the last refuge of the Mongolian Saiga (*Saiga borealis mongolica*), a distinct endemic subspecies of *Saiga borealis* that once roamed from the British Isles through Central Asia and the Bering Strait into Alaska and the Yukon. The status of the Saiga improved significantly during the last 3 years' project; however its long term survival is still threatened by habitat degradation caused by livestock. High numbers of livestock and unsustainable pastureland management continue to lead to habitat degradation, overgrazing and competition between livestock and wild ungulates. Current pastoral land-use is a downward spiral of decreasing herder mobility and increasing out-of-season grazing, leading to unsustainable use of grassland. This trend of heavy competition between wildlife and livestock for open water and grazing areas is ongoing, despite some progress in the Saiga distribution. WWF Mongolia is working with herders to improve rangeland management by linking Saiga conservation to alternative income activities. Herder groups are established and motivated to solve problems on pasture degradation which in return provides benefits for the Saiga and other wildlife. However, it took much more time then expected to establish functioning herder groups. More time is needed to turn this into a long-term success throughout the Saiga range.

2-3. Predicting Plant Species Diversity and Changes in Productivity Due to Simulated Effects of Cattle Disturbances in Japanese Grasslands

Yu YOSHIHARA

Tohoku University, Japan

Grazed pastures in Japan historically have been used for animal production with little thought given to biodiversity, despite many endangered species depending on this habitat. Pasturing has both positive biodiversity and negative productivity impacts as a result of gaps in the distribution of vegetation caused by cattle activities. We therefore attempted to predict plant species diversity and changes in productivity from simulations of cattle activities at three pastures with different vegetation communities. We simulated three ground types (spatial proportion of grazed land, bare ground, and undisturbed areas) in three pastures and recorded the percentage cover of each plant within plots. We recalculated these community data by varying the sampling probabilities and sampling numbers, which provided us with species diversity indices at multiple scales. We also investigated dry mass and forage qualities by chemical analysis. For an improved pasture and partially improved semi-natural pasture, our models predicted that plant diversity increased as a saturating function of the proportion of bare ground and grazed area to the undisturbed area, but our models also showed exponential curves in the semi-natural pasture, irrespective of scale. Productivity (dry mass × forage quality) was comparable between undisturbed and grazed plots, except in semi-natural pasture. Forage samples on bare ground plots had the lowest productivity across all pastures. From the predicted effects of cattle pasturing on both biodiversity and productivity, maintaining a small proportion of bare ground (20-30%) in the improved pasture coinciding with extensive grazing (70-80%) is a more practical application.

2-4. Effect of Grazing on Habitat and Behavior of Wild Mammals

Hideharu TSUKADA

National Institute of Livestock and Grassland Science, Japan

Although there are few grassland specialists among terrestrial mammal species (110-115) in Japan, 52 mammal species inhabit Japanese grasslands and 22 of them are listed in the RDB of Japan. However, some grassland mammals, such as the sika deer and the wild boar cause serious damage on grasslands. The relationship between cattle grazing and wild mammals in Japanese grasslands should be considered from the following two contexts: 1) conservation of grassland mammals and 2) the control of mammal damage in grasslands. Firstly, the effect of cattle grazing on the habitat of small mammals was considered in the context of conservation of grassland mammals. The habitat quality for small mammals was high in tall-grass pasture with low grazing intensity but low in short-grass pasture with high grazing intensity. Therefore, there is a trade-off between the habitat quality for small mammals and the productivity for grazing cattle in the animal production system. Secondary, zoning with grazing cattle between wildlife habitat and agricultural area was considered for controlling wildlife damage in grasslands. In the abandoned cultivated land, the introduction of cattle grazing was effective deterrent against rooting by wild boars because cattle grazing had diminished the food resource and the secure cover for wild boars. However, the intrusion of sika deer into pastures could not be prevented by cattle grazing. The activity of sika deer within a stock farm was affected by the nearest escape cover and the herbage quality. Hence, grazing cattle alone is not sufficient for preventing wildlife damage in grassland.

2-5. Forage Production, Utilization and Environmental Conservation in Sweden

Halling MAGNUS

Swedish University of Agricultural Sciences, Sweden

There are totally 1.5 million ha of grassland in Sweden of which 70 percent is temporary in rotations on arable land. Temporary grassland is the dominating crop with 40 percent of the arable cropping area. The climate is cold temperate and winter damages are common in the grasslands. In the north, where farming is done up to 66 degrees latitude, light conditions are extreme, which gives higher energy content in the forage. Timothy (*Phleum pratense* L.), meadow fescue (*Festuca pratensis* L.) and red clover (*Trifolium pratense* L.) are the dominating species. Contrary to the intensive grasslands in Europe, it is very common to use a mixture with grasses and legumes. Silage maize can only be grown in very south where the temperature sum is high enough. The area of silage maize is rapidly increasing due to new varieties. Larger milk yield per cow increase the requirement for herbage with higher nutrient quality. This has lead to earlier cuts and increasing number of cuts. Due to the long winter season a large part of the forage is conserved as silage. In 2009, Sweden had about 350 000 dairy cows on nearly 6 000 farms. About ten percent of the milk farms have organic production. Efforts to reduce N-losses to the environment are done by develop grazing systems and total feeding rations that increase N utilization. Also slurry injection into grassland has advantages over slurry spreading as it decreases ammonia losses and odour and improves forage hygiene.

3-1. Animal Wastewater Treatment Using Constructed Wetlands

Chika TADA

Tohoku University, Japan

Data collected in December 2009 indicate that there are about 1 million livestock farms in Japan that must abide by the nation's effluent standards. A 2007 survey counted about 650 dairy farms. Wastewater from dairy farming is usually treated aerobically, but the aeration consumes large amounts of energy. Recently to achieve KYOTO Protocol requires the reduction of carbon dioxide emissions from wastewater treatment systems. Constructed wetlands have receives attention as energy-free and eco-friendly wastewater treatment systems. In this study, constructed wetlands were hybridized with vertical and horizontal flow systems to treat wastewater from a milking parlor. Water quality was investigated by determining the number of coliform bacteria and ammonia-oxidizing microbes present during the treatments. We were able to remove more than 90% of COD, TN, and TP from raw wastewater in June through September. However, these removal rates decreased after October, with the lowest removal observed in December. Real-time PCR analysis found 10^2 to 10^4 cells/cm^3 ammonia-oxidizing bacteria and 10^2 cells/cm^3 ammonia-oxidizing archaea. However, ANAMMOX bacteria were not detected in December. The removal rate of coliform bacteria did not differ from September to December, except on a single day. Furthermore, no significant differences in treatment efficiency among the three types of wetlands were observed. In particular, we found no difference in the removal rate of coliform bacteria among constructed wetlands, with or without plants. The most efficient removal of coliform bacteria was observed on October 22, following 3 days of sunny weather. This observation suggests that soil-drying due to the absence of influent was important for removing coliform bacteria. Regulations require that COD and TN concentrations in treated water that is to be reused for agriculture be less than 6 mg/L and 1 mg/L, respectively. In addition, reclaimed water for landscape use must contain fewer than 1000 cfu/100 ml coliform bacteria. The concentration of COD, TN, and coliform bacteria in the wetland-treated water in summer met these standards for agricultural and landscape reuse. In the future, further improvement of removal efficiency in constructed wetland treatments will allow more animal wastewater to be reused. In addition, a recycling system including the utilization of treated water must be established and promoted.

3-2. Introduction of Biomass Accounting as an Evaluation Tool of Biomass Utilization Systems: a Case Study of Domestic Animal Waste Treatment-Oriented Biomass Town

Lyudmyla BESPYATKO, Kiyotaka SAGA, Takashi YANAGIDA, Win AUNG, Shinji FUJIMOTO and Tomoaki MINOWA

National Institute of Advanced Industrial Science and Technology, Japan

Traditionally, primary industry's biomass leftovers had been treated with the purpose of waste reduction. However, nowadays, efficient utilization of biomass resources is regarded as an important tool of rural economy revival, zero-emission society establishment, and reduction of greenhouse gas emissions. In Japan, the necessity of biomass utilization for sustainable society was declared in Biomass Nippon Strategy (2002). Based on this document, many rural towns and villages all around the country has adopted a "Biomass Town" concept and started to develop biomass utilization systems with multiple activities, including material, energy, and education biomass-related projects. However, no comprehensive evaluation of economic and environmental efficiency of already existing biomass utilization systems is conducted. One of the reasons is that there exist no guidelines, which could assist in such estimation process. In this paper, first, brief introduction on biomass accounting concept, suggested by authors in our previous works as an accounting tool to assist in decision-making related to biomass activities, is made. Next, the results of a case study on domestic animal waste treatment-oriented biomass town are presented. The biomass accounting form is applied in the case study to account for four compost facilities built and managed by local government. Finally, discussion on the possibility of economic and environmental efficiency improvement of the biomass activities under the case study is conducted.

3-3. Power Generation from Animal Wastewater Using a Single-chamber Microbial Fuel Cell

Osamu ICHIHASHI

Oak Ridge National Laboratory, USA

Microbial Fuel cells (MFCs) are bioreactors, which generate power directly from dissolved organic substrate, such as sugars, organic acids and biomass by using electrogenic organisms as biocatalyst. The concept of MFCs has been known since about 100 years ago, however it hadn't attracted much attention because current and power production was very small. For these 10 years, the current and power production of MFCs has been advanced significantly, and MFCs has attracted attention as a sustainable power production technology. Especially in the field of wastewater treatment, practical application of MFCs is strongly desired because MFCs can achieve both sanitization of water and power generation at the same time. Furthermore, wastewater theoretically contains several times higher potential energy than the required energy for its own treatment. Except MFCs, there already have existed wastewater treatment processes which can achieve energy recovery such as methane fermentation. However, compared to methane fermentation, MFCs have some advantages. In the field of animal industry, the treatment of great amount of animal manure has been a big problem because it requires a lot of energy. However, to say reversely, animal manure contains great amount of potential energy. The increase of construction of methane fermentation plant shows the high interests in the energy recovery in this field. In this presentation, recent trial of MFCs, mainly animal wastewater treatments are reviewed.

3-4. Archaeal Community during Cattle Manure Composting Process in Field-scale Facility

Nozomi YAMAMOTO

Tohoku University, Japan

Composting process is a useful technique to transform cattle manure into organic fertilizer. During the process, complex microbial communities consisting of bacteria, archaea, and fungi carried out biodegradation of substrates. Because it has been considered that bacteria play an important role in composting, their community structure was studied well. However, archaeal one has not been identified clearly. To understand their community structure and abundance, cattle manure was composted in field-scale facility and composting materials were analyzed by culture-independent approaches. Clone library constructed from archaeal 16S rRNA genes showed that archaeal community in compsot was mainly consisted of methane-producing archaea (methanogen) and ammonia-oxidizeing archaea (AOA). During first 2 days, clones which were related to methanogens in the rumen or intestine were detected, suggesting that fecal methanogen could survive in the early stage of composting. Other methanogen, which grouped into thermophilic *Methanosarcina* spp. were present thoroughout the process, indicated that they might adapt the environmental changes such as high/low temperature. For the first time, AOA sequences were detected from composting materials. They showed high identity (98%) with cultured AOA originated from hot spring. The number of the archaeal genes from AOA were nearly same as or slightly higher than that of bacterial gene from ammonia-oxidizing bacteria (AOB), which initially considered the sole autotrophic ammonia oxidizers in the environment. In this study, we revealed the changes in archael community in the composting process. It was also suggested that not only AOB but AOA could actively involve in nitrification of composting systems.

3-5. Development of a New Fermentation Process for Hardly Degradable Rice Straw Agriculture Waste

Yingnan YANG, Rui ZHAO, Zhenya ZHANG and Norio SUGIURA

University of Tsukuba, Japan

In order to develop the new and high-efficiency methane fermentation process for hardly degradable rice straw, the co-digestion performance of different amount of rice straw and sewage sludge was investigated. The digestion of rice straw and sewage sludge under the investigated condition resulted in VFA accumulated in the digester. The influences of temperature, moisture content and initial pH value on VFA accumulation in the hydrolysis step of methane fermentation were tested. The results showed that the total VFA concentration was increased with initial pH value, and was decreased with temperature and moisture content. Then the feasibility of rice straw pretreatment with the accumulated VFA from the fermentation process was studied. For the pretreatment process, the increase of acid concentration, pretreatment time and ratio of solid to liquid in some extent increased the lignin reduction and the hydrolysis rate. About 34.19% of lignin was removed and 21.15% of the hydrolysis rate was obtained in commercial reagent pretreatment by 2.5% of acid for 2 h at the ratio of 1:20 (w/v), and 10.7% of weight loss was obtained by pretreatment with drip washing VFA solution. The production of methane gas from rice straw was significantly enhanced as nearly as two times by drip washing VFA solution pretreatment than that of untreated rice straw. This suggested that the accumulated VFA from the rice straw fermentation process can be used as a kind of pretreatment reagent for improving biodegradability and methane production. So a low-cost and high-efficiency methane fermentation process for hardly degradable lignocellulosic materials such as rice straw could be established.

Poster Session

1. The Effect of Brushing by a Human on Behavioral and Physiological Stress Indicators of Cows

Chiaki AKASAKA, Shigeru NINOMIYA and Shusuke SATO

Tohoku University, Japan

In the previous study, we revealed that steers used regularly the fixed brush to walling for self-grooming for nine months. They produced higher valued guts and dressed carcasses than those reared in a pen without a fixed brush. In order to investigate the effect of brushing, behavioral and physiological stress indicators were assessed in cows during being brushed by a human.Effective body regions brushed by a human were investigated in six Japanese black cows: loin, belly, neck and head. Behavioral reactions and heart rates were recorded during being brushed for ten days. The head and loin were revealed as the highest and the lowest effective body regions to being brushed, respectively, in this experiment. These regions were brushed in each cow for three min per day for three days. The behaviors of six cows after brushed were recorded to assess the calming effect of brushing. Then oxytocin and cortisol concentrations in plasma and heart rates in three cows were monitored before, just after, 3, 15, and 30 min after brushed under tethering. On the 10th day, heart rates of cows during being brushed of necks were lower than of bellies ($p<0.05$). Resting behavior tended to increase being brushed. Plasma oxytocin concentration tended to increase just after being brushed of head. Plasma cortisol concentration was constant during 30 min after being brushed of heads and loins under tethering, while that not being brushed was significantly higher after tethering ($p<0.01$).

2. The validity of Salivary-amylase as Stress Marker of Cattle

Akane KITAGAWA, Chiaki AKASAKA, Shigeru NINOMIYA and Shusuke SATO

Tohoku University, Japan

Non-invasive and simple methods are required on on-farm welfare assessment. Thus salivary measures are focused as an example of non-invasive stress marker. The aim of this research is to assess whether salivary-amylase is effective as stress marker of cattle. Test1 To examine diurnal variation of salivary-amylase, saliva from 7Holstein heifers were collected by using surgical clamp with cotton across 2days at the 12times (6, 9, 12, 15, 18, 21 o'clock). Heart rate monitor was attached heifers at the same time, to investigate relationship between salivary-amylase and heart rate.Test2 We divided 7heifers into 2groups according to with (NR) or without (CR) of nose-ring both group were restrained with rope. Saliva from heifers was collected before and after wearing nose-ring or restraining treatment at total of 8times and thus salivary-amylase and cortisol levels were estimated. Heart rate monitor was attached heifers. The average of salivary-amylase levels was 68.9kIU/l±33.9 and was independent of the time and day. There was no difference between NR and CR in the salivary-amylase, cortisol levels and heart rate. Salivary-amylase levels after restraining were significantly higher than before restraining with rope. Salivary-amylase level was significantly correlated with heart rate. In conclude, salivary-amylase is effective as stress marker of cattle.

3. Overall Animal Welfare Assessment of Dairy Cattle at the Farm Level Using the Shinshu Comfort Livestock Farm Certification Standard

Ai MORIMOTO and Kenichi TAKEDA

Shinshu University, Japan

The "Shinshu Comfort Livestock Farm Certification Standard," is an overall welfare assessment model for farm animals, which was proposed in 2007 in Nagano Prefecture, Japan. However, the usefulness of this model at the farm level has not been evaluated. We evaluated the overall animal welfare of dairy cattle at the farm level using the assessment methods and investigated the usefulness of these assessment methods. Fifteen dairy farms employing one of three different housing types (tie-stall system, free-stall system, and grazing system) were visited, and welfare levels of all cattle reared on these farms were assessed over summer. The assessors scored the welfare levels using the assessment methods of the Shinshu Comfort Livestock Farm Certification Standard. This model contains 65 measures based on the following "Five Freedoms": (1) freedom from injury, or disease; (2) freedom from hunger and thirst; (3) freedom from discomfort; (4) freedom to express normal behavior; and (5) freedom from fear and distress, in terms of three bases (animal, management, and environment). Total scores ranged from 301.2 to 397.7. Average values (± SD) of tie-stall, free-stall, and grazing systems were 345.5 ± 13.3, 393.9 ± 4.3, and 351.4 ± 35.8, respectively. Furthermore, total scores varied markedly among the farms and the grazing system did not necessarily score the highest. The category score for Freedom 1 was higher in the free-stall system than in the grazing system ($P < 0.05$) and that for Freedom 4 was higher in the grazing system than in the tie-stall system ($P < 0.01$).

4. The Effect of Environmental Enrichment on Welfare and Productivity in Homebred Strain "Tatsuno"(Chicken for Meat)

Ai OHARA[1], Mitsuhiro TANIMURA[2] and Shusuke SATO[1]

[1]Tohoku University, Japan, [2]NFF Co.,Ltd.

Environmental enrichment of RSPCA standards are known as effective rearing system for animal welfare and economic efficiency. We provided perch and bales recommended by RSPCA to birds at commercial farm (NFF). Behaviour, H/L ratio, air quality, breast blisters and foot-pad dermatitis(FPD) were surveyed as indicators of welfare, and final body weight and feed conversion were measured as indicators of productivity. This study used 4 flocks (male or female×enrich or control) of about 5400 birds. Environmental enrich treatment flock had 30.8m perch and 8bales. We surveyed maintenance behaviour (eating, drink, sitting-rest, standing-rest, locomotion), perching, bale pecking and air assessment at 3/5/8 weeks of age. Blood samples were collected from 10 birds/flocks at 20/45/56 day of age. Breast blisters and FPD were counted for 200 birds/flocks at slaughter house. Birds in the enriched house (E flock) were more active (showing more standing-rest and locomotion). Male birds like bales pecking, female birds like perching. Final body weight and feed conversion of E flock were larger and good than C flock. The number of bird with non-breast blister on E flock was higher on male, and the number of birds with FPD of E flock was low grade lesion on female. In conclusion, there are differences between male and female preference of activity. It might influences productivity. These findings may have implications for develop rearing system according to behavioural needs.

5. Is the Dairy Cow Performing to Bite Grasses Comfortable in Grazing?

Chisako OYAKAWA, Kazuya SATO and Shusuke SATO

Tohoku University, Japan

The grazing behavior of cattle can divide into two characteristic phases. The cattle look for grasses on the pasture, and then, they eat the standing grasses by manipulating their tongue complexly, which are called as “searching phase” and “biting phase”, respectively. However, cattle in the housing system could not express these behaviors normally. Since cattle are fed mowed grasses from the trough in the housing system, they need not to look for favorable grasses and to manipulate tongue for eating. From the viewpoint of animal welfare as the freedom to express normal behavior, they might be in psychological stress. In the present study, we examined whether the biting phase induces the cow to be comfortable. Ten dairy cows were used in this study: 5 cows had been reared in the grazing system and another 5cows had been reared in the housing system. They were individually exposed to each of 2 feeding situations: 1) a cow grazed at the pasture sized 0.7m×4.3m under loosely confinement, 2) a cow fed mowed grasses at the trough sized 0.7m×4.3m also under loosely confinement. After 10 min feeding, blood from the jugular vein of a cow was collected for assessing the oxytocin and coltisol concentrations in plasma as indicators of comfort and stress, respectively. In both groups, there were no significant differences between 2 feeding situations on the concentrations of oxytocin and cortisol of cows. Thus, we conclude that the effect of performing only the biting phase in the grazing behavior might be little for inducing comfort of cows in the grazing situation.

6. Does Housing Conditions Affect the Flight Distance of Dairy Cows?

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The human-animal relationship is an important factor when considering animal welfare. The flight distance, that is how close a human can come to a stationary animal before it moves away, is an indicator of the relationship. However, the distance may varied under restrict housing conditions. We investigated that the flight distance of dairy cows in different housing condition. Eighteen lactating Jersey cows were used and usually tethered with 55cm chain in stalls at night and a pasture in the daytime. Flight distances of these cows were measured three times under the tie-stall housing condition and grazing condition. Under the tie-stall housing condition, standing cows were approached slowly (one step per sec) from the front by the experimenter (1.64m tall), who held the arm overhand in an angle of the 45˚ in front of the body. The distance between the experimenter's hand and the focal cow's head was estimated at the moment of the cow's withdrawal according to Waiblinger et al (2003). Under the grazing condition, the same experimenter approached slowly (one step per sec) standing cow's front. The experimenter stopped walking when the cow stated moving away. The distance from experimenter and where the cow moved was estimated with eye according to Rousing et al (2004). The flight distance score under the tie-stall housing condition was significantly and positive correlated with under the grazing condition ($r_s = 0.70$, $P<0.05$). The result suggests that the flight distance score of a cow is the same even if cow had been tethered.

7. The Linkage Between Pastoralists' Perspectives and Vegetation Threshold Changes in Mongolian Rangelands

Kaoru KAKINUMA[1], Takehiro SASAKI[2], Tomoo OKAYASU[1], Undarmaa JAMSRAN[3], Toshiya OHKURO[1] and Kazuhiko TAKEUCHI[1]

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Rangelands in semi-arid regions are systems in which human activity and nature are tightly linked. Previous studies have suggested that pastoralists prefer opportunistic movement to access highly variable resources associated with high rainfall variability rather than regular movement, and vegetation threshold changes along grazing gradients have been observed across Mongolia. It is therefore important to understand how pastoralists perceive vegetation threshold changes. Here, we test the hypothesis that the pastoralists living in environments with higher rainfall variability have negative perceptions of vegetation threshold changes, whereas pastoralists living in environments with lower rainfall variability have positive perceptions of them. The study areas were steppe and desert-steppe in Mongolia, desert- steppe were relatively higher rainfall variability than steppe. We performed a vegetation survey in each area and interviewed pastoralists along grazing gradients. Our result showed that pastoralists in the desert-steppe area perceived the post-threshold vegetation state negatively, whereas pastoralists in the steppe area perceived them optimistically. We suggest that, although the observed ecological patterns were similar, the pastoralists' perceptions of them were influenced by environmental context, which has important implications for sustainable rangeland management.

8. Recovery of *Pleioblastus variegatus* from Flowering in the Aso Region -Results of the 17th Year-

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National Agricultural Research Center for Kyushu Okinawa region, Japan

Miscanthus-Pleioblastus type grasslands is typical vegetation in the semi-natural grasslands of the Aso region, and the grassland has been historically managed by grazing and mowing in summer and burning in early spring. However, in 1992, a phenomenon occurred whereby *Pleioblastus variegatus* flowered all together and died within a wide range of West Japan. In the Aso north somma, the withering area extended to 2,150ha. No previous reports have described the process of recovery of *Pleioblastus variegatus* in the Aso region. In this study, to investigate the effect of grazing on the recovery of *Pleioblastus variegatuwe* on the semi-natural grasslands in which *Pleioblastus variegatus* had dominated before it flowered, we compared the grazing area and the grazing prohibition area. An earlier paper (Ootaki and Nasu,1995) described the growth of *Pleioblastus variegates* seedlings, which exhibited slow recovery due to the dominance of other species (*Miscanthus sinensis* and *Arundinella hirta*) at the second year after flowering. By the 17th year, *Miscanthus sinensis* was the most dominant species in both the grazing area and the grazing prohibition area. *Pleioblastus variegatus* was the next dominant species in the grazing area, and the rate of coverage of *Miscanthus sinensis* and of *Pleioblastus variegatus* were 56.7% and 25.3% respectively. In contrast, in the grazing prohibition area *Pleioblastus variegatus* did not appear among the top 10 dominant species. Therefore, we conclude that grazing hastens the recovery of *Pleioblastus variegatus* in the Aso region.

9. Effect of Disturbance Size on Similarity and Resilience of Vegetation to the Disturbance

Miya OKADA, Yu YOSHIHARA and Shusuke SATO

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Cattle create disturbance patches of varied sizes in sloping pasture through their activities (grazing and trampling). We investigated the effect of disturbance size on similarity and resilience of vegetation to the disturbances. This study was carried out at three vegetation types (improved pasture, partially improved semi-natural pasture and semi-natural pasture) in Kawatabi Research Station of Tohoku University, Japan. We created diameter 20, 40, 60 and 100cm of trampling (removal of soil surface at 5cm deep) and grazing (mowing at 5cm height) treatments in mid-May, late June and early August. A 100 ×100cm undisturbed treatment (control) was set in each plot. Similarities of species composition between the disturbance sizes and control were calculated from the species compositon in the one month after the disturbances in each season. In all vegetation types, a similarity between 20cm and control was the smallest in each combination . Species composition of grazing treatment was relatively similar to that of control. Resilience of vegetation at 20cm and 100 cm of tramplimg treatment created in May were shown by continuous resultant cover from May to September. Resilience tended to be grater at 100cm than 20cm of trampling treatment, in the improved pasture and partially improved semi-natural pasture (p values = 0.09, 0.08, respectively). Also, resilience of the semi-natural pasture was significantly grater at 100cm ($p < 0.001$). We suggest that small disturbance size had a profound effect on the similarity and resilience of vegetation, probably because surrounding vegetation shaded the sun.

10. Quantification of Bite Size, Bite Rate and Intake Rate in Cattle Foraging Japanese Native Grasses

Hajime TOMIMATSU, Misa YOKOYAMA and Shin-ichiro OGURA

Tohoku University, Japan

Bite size (BS) and bite rate (BR) are fundamental elements relating intake rate of grazing animals. In this study, an indoor experiment was conducted to quantify BS and BR, and their change with decrease of availability in *Sasa palmata* and *Miscanthus sinensis*, which are major species in Japanese native grasslands. These grasses were harvested immediately before the experiment in early summer. Twenty-five shoots of *S. palmata* (60 cm) and *M. sinensis* (40 cm) were planted in a planter at a 10 cm interval, and offered in front of four cows separately. The number of bites was counted until all the shoots received at least one bite (Maximum bites; MB), and 1/2 MB, 1/4 MB, and two bites. Amount of intake was measured by dry weight loss during the foraging periods. MB of the cows was 26-40 and 34 bites in *S. palmata* and *M. sinensis*, respectively. BS in *S. palmata* (1.34-3.20 g DM/bite) was greater than in *M. sinensis* (0.83-1.75 g DM/bite). In both species, BS was highest in the first two bites. BR in these grasses ranged similarly; 27.2-38.3 and 25.8-40.0 bites/min in *S. palmata* and *M. sinensis*, respectively. Responding to these biting behavior, intake rate was highest in the first two bites (*S. palmata*, 148.6 g DM/min; *M. sinensis*, 61.0 g DM/min) compared to the longer foraging periods (33.8-37.5 and 23.0-39.7 g DM/min). The results suggest that BS is a major factor affecting intake rate in *Miscanthus* and *Sasa* dominant grasslands.

11. Do Cattle Choose Preferred Plant Species Without Visual Sense?

Misa YOKOYAMA, Masatoshi HORI and Shin-ichiro OGURA

Tohoku University, Japan

It is known that volatile flavor compounds (VFCs) released from fresh leaves are different among plant species. We recently showed great difference of relative preference (RP) by grazing cows and VFCs among Japanese native plants; *i.e.*, *Miscanthus sinensis* (high RP, major VFC was green leaf volatiles), *Sasa palmata* (medium RP, sesquiterpenes) and *Pteridium aquilinum* (low PR, C_8 compounds). In this study, feeding experiments were conducted to examine whether cattle choose preferred plants by using VFCs as cues in food choice without visual sense. Five Japanese Black beef cows were used after taming the experimental condition. Fresh leaves of the three native plant species were harvested two hours before the experiments, and two among the three species were simultaneously fed with 30 cm apart, close to muffle of each cow. This operation was repeated 10 times per cow firstly under non-blinder (NB), then blinder (BL). The cows chose *M. sinensis*, *S. palmata* and *P. aquilinum* in 87, 62 and 0 occasions, respectively, out of 100 in NB ($P<0.05$). Similarly, they chose *M. sinensis*, *S. palmata* and *P. aquilinum* in 79, 67 and 0 occasions, respectively, in BL ($P<0.05$), indicating the cows chose the preferred plant without visual sense. However in BL, the cows touched *P. aquilinum* with their muffle in 10 occasions, and put it in their mouth in 13 occasions. These results suggest that scent plays a major role in discriminating foraging plants, and visual and tactile sense also function food choice of grazing cattle in Japanese native pastures.

12. Analysis of Bioconversion and Bacterial Community of Thermophilic Hydrogen Production Using UASB Reactor

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To solve the current energy issue, hydrogen has attracted with a great attention. The hydrogen gas can be produced by fermentation with organic waste, and the energy dose not release carbon dioxide. Thus the hydrogen is considered to become a one of sustainable and useful energy source. From previous studies it has been supposed that applying a UASB (Up-flow Anaerobic Sludge Blanket) reactor widely used for anaerobic wastewater treatment for hydrogen fermentation enables to perform effective biological hydrogen production, because produced granular sludge which work as a reacting field is produced in the reactor. In this study, we examined hydrogen production with UASB process under thermophilic condition. Starch wastewater. During the process, we analyzed a transition of biochemical conversion and bacterial community structures in the granular sludge. In this analysis, a UASB reactor equipping an external water jacket was used. The working volume of the reactor was 6L, and the temperature was controlled at 55˚C. Starch was used as a sole substrate (15 g-starch/L). The reactor was operated for 86 days, and the HRT was decreased stepwise from 24h to 12h to increase its substrate concentration. Bacterial community in the granular sludge was estimated with 16S rRNA gene cloning analysis. During our operation, hydrogen content in processed biogas was in the range of 28.6 - 38.3%, and the pH value conversion of effluent was 4.6 - 5.3. The maximum yield of hydrogen production was 1.20mol H_2/mol glucose and was observed at HRT24h. At 24hHRT, butyric acid was dominant in the metabolite. But lactic acid was increased and butyric acid was decreased, when the HRT was shorter than 24h. In the bacterial community, *Thermoanaerobacterium*, which is known as a hydrogen producing bacterium, was dominated. From FISH observation, *Thermoanaerobacterium* was predominant at 24h HRT, but its population size was decreased at 16h HRT. The shifting of bacterium population might be caused by conversion of metabolite composition.

13. Methane Fermentation from Waste Glycerol and Sludge ~Approach on Miyagi Prefecture 3 R New Technical Research Development Project~

Yasunori BABA, Ryoya WATANABE, Chika TADA and Yutaka NAKAI

Tohoku University, Japan

Glycerol of biodiesel manufacture process and wastewater sludge of food factory were digested by methane fermentation. This project is supported by 3R project of Miyagi Prefecture. The glycerol and the wastewater sludge were produced in Osaki city, north part of Miyagi prefecture. Until now, these wastes have burned or composted as waste treatments. In the present study, the energy recovery from methane fermentation of the glycerol and the wastewater sludge was estimated. The digested sludge from the methane fermentation was assumed as liquid manure. The best fermentation condition was addition of 0.5%/day glycerol to the volume of sludge containing seed sludge and the wastewater sludge (5% of dry weight) in the proportion of one part to three. Production of methane was 48.4 ml per 40 ml of the mixture. The digested sludge derived from the methane fermentation includes 98% of the nitrogen, 5% of the phosphorus and 2% of the potassium required to grow rapeseed in 1ha. Provisional calculation of EPR (Energy Profit Ratio) were 0.25. However, the social system introduced the methane fermentation would get higher energy production than that without the methane fermentation. Therefore, methane fermentation will become a help of the creation of a recycling society. The pretreatment method of the sludge is examined for the improvement of the methane yield now.

14. Methane Fermentation Using Waste Glycerol Produced from Biodiesel Fuel

Ryoya WATANABE, Yasunori BABA, Chika TADA and Yutaka NAKAI

Tohoku University, Japan

Waste glycerol is generated from the fuel manufacturing process as a by-product. It is expected that the biogasification by methane fermentation is effective as one of the recycling of waste glycerol. There are some reports for the processing performance of methane fermentation using the glycerin, but in these reports the additive amount of the waste glycerol becomes a problem. The purpose of this study is that the possibility of effective use for waste glycerol of methane fermentation is examined with batch reactor. The waste glycerol was added to vial bottles (100ml) containing seed sludge used of cattle manure (10ml), and activated sludge from the noodle manufacturing factory (30ml). The additional condition of the waste glycerin is 0, 0.25, 0.5, 1.0, and 2.0%/day. The temperature was maintained at 35˚C, with shaking for 18 days. In each condition, composition of biogas and amount of biogas production, COD and VFA were measured. Biogas productions from the 1.0 %/day and 2.0 %/day glycerol addition were higher than the other until second days of starting operation, but gradually decreased as the time passed. On the other hand, biogas production from the 0.25%/day and 0.5%/day was gradually increased. Amount of biogas production from the 0.25%/day glycerol addition was constant. The accumulation of biogas production from the 0.5%/day glycerol addition was the highest in all conditions. The methane gas concentration at this time was about 30% that was lower than 60% of general methane fermentation. About 60% COD of glycerin were degraded under the 2%/day glycerol addition, because VFA remained. Degradation of butyric and valeric acids were stopped in all conditions except the 0%/day glycerol addition.

15. Prevalence and Distribution of Bovine Coccidia in the Northern Area of Miyagi Prefecture

Rintato SATO, Fumi MURAKOSHI, Yasuhiro FUKUDA and Yutaka NAKAI

Tohoku University, Japan

A coccidiosis, which is caused by parasitic protozoa Eimeria spp., is one of the most popular infectious diseases in various kinds of domestic animals including cattle. Bloody stool and diarrhea are typical symptom of coccidiosis, and infected animals are sometimes killed in severe cases. Moreover, in recent years, some cases observed drug resistant capability were appeared, and the toxicity of bovine coccidia has been increasing. Thus, the coccidiosis has been feared to decrease the productivity and to damage a vast economic loss in the world livestock industry. In this study, to clarify the infection state of bovine coccidia, we investigated four farms in the northern area of Miyagi prefecture. One hundred eighteen cattle from three dairy farms and 108 beef cattle from different one farm were analyzed by rectal examination. In three dairy farms, infection ratio of coccidia indicated 11.5% and low value, and the ratio tended to be high in young adult cattle. In contrast, very high infection ratio (76.7%) was obtained from beef cattle in one farm, but conspicuous sympotom of coccidiosis was not observed from such cattle. From previous study performed in 1986, the ratio of coccidium infection was 19.3% in the same farm showing the highest ratio in this present study. This result indicates that subclinical infection with some bovive coccidia that possess low toxicity could be occued easely, and that a risk of outbreaks might be expanded without our awareness.

16. Cattle Is Possible Host Animal of The Land Leech

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We examined the joint effects of density dependence, climatic variation and hatchery release on the population dynamics of land leech. Host animals of the land leech Haemadipsa zeylanica var. japonica were investigated by collecting 46 leeches in 2009 at stock farm in Kanagawa prefecture, and a number of mammals indigenous to the forests sample were analyzed by PCR-single strand conformation polymorphism (SSCP) method of 28S rRNA genes. These results indicate that cattle aid in the propagation and spread of leeches at stock farm. The land leech population in Akita prefecture was the northernmost on Honshu Island, Japan. However, the number of land leech and areas of habitats were rapidly expanded to Iwate prefecture by sika deer, Japanese serow, and human (forest workers, farmer and hikers). In order to solve these problems, control of stock farm, wild life population (such as sika deer) and forest environmental are needed.

17. Availability of Forest Resources as Firewood for Preservation of Regional Environment

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We studied the availability of the forest resources as the firewood in the region. Recently, people have not cared for the forest, and consequently the numbers of wild animals and edible wild plants have decreased. Using the forest resources is one of the important ways to maintain the balance among people, wild animals and environment. The study site was the Ooisawa district, Nishikawa town, Yamagata prefecture in Japan. We investigated the usage amount of firewood by a questionnaire and the available forest area by using remote sensing and GIS from August in 2009 to February in 2010. The number of households in this region is 113 and 36 households use woodstoves. The average amount of annual usage of firewood was 6 t. If all of the households in this region use the woodstove for heating, the requisite amount of firewood was 678 t per year. Assuming the harvest at the cycle of 30 years, the firewood for all the households can be provided sustainably with the broad-leaved trees within 100 m from the road described in the topographic map on a scale of 1/25,000.

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