# Behavior and Ruminal Characteristics of Japanese Black Cattle Grazing in Forest of Young Tree Plantation and Native Grassland

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## Abstract

Three of Japanese Black female cattle were grazed in a young forest area of *Chamaecyparis obtuse* plantation (YF, June to September in 2003) and in a native grassland area (NG, October and November in 2003). Twenty-four hour behavioral observation of the cattle was conducted and a GPS was used to determine the distance traced by the cattle once a month during experimental period. The rumen fluid was collected twice in each area on early and late set-stocks. Grazing time averaged 537.7±109.8 min/day and was positively correlated with the rate of grazing *Miscanthus sinensis* ($p<0.05$) and negatively correlated with the rate of grazing *Pleioblastus simonii* ($p<0.001$). The rate of grazing *M. sinensis* was negatively correlated ($p<0.05$) with the rate spent grazing *P. simonii* and other plants ($p<0.001$) and positively correlated ($p<0.05$) with the rate of lying posture. The rate of grazing other plants was negatively correlated ($p<0.05$) with rumination time. Distance covered by cattle ranged from 5001m to 6879m through experimental period. Differences in total VFA were slight, however, differences in individual VFA's were seen with area as well as set-stock time. $NH_3$-N concentrations were higher in YF (6.4 mg/dl and 7.2 mg/dl) than in NG (4.5 mg/dl and 4.3 mg/dl). Total protozoa counts per ml decreased from $2.0 \times 10^6$ (early set-stock)to $3.0 \times 10^5$ (late set-stock) in YF, whereas in NG remained the same at $1.0 \times 10^6$. *Entodinium* was found in highest percentages in both areas. Total bacteria counts/ml was ranged from $1.4 \times 10^7$ to $8.2 \times 10^8$ and gram negative cocci and gram positive cocci bacteria were found in high percentages. In this study, it is shown that cattle is able to adapt to the young forest and native grassland by changing the behavior and selecting plants from the diverse vegetation.

Key words:grazing behavior, Japanese Black cattle, young forest, native grassland, rumen fluid

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## INTRODUCTION

Grazing of beef and dairy cattle on native lands has been done extensively over the years in rural areas of Japan as a means of providing forage for cattle. Due to reasons such as low animal production[7,8], this system is not considered as efficient as sown pastures. Japan is characterized by a high proportion of forests and woodland (66%)[18], which have the potential for animal grazing and production areas. The livestock production from forest and woodlands could therefore be improved by grazing in a way that took advantage of the changes in forage quality and availability[9]. Hence it is important to study the behavior and nutritional status of the cattle set-stocked on these lands, in order to develop a sustainable and effective grazing system. Ruminants are able to utilize forages because they posses microorganisms in their rumen, which release enzyme to digest the cell walls[10]. Until now, limited information is available on intake, digestion and rumen fermentation patterns of cattle grazing in native lands. This study examines behavior and rumen characteristics of Japanese Black cattle set-stocked in a young forest and mative grassland areas.

## MATERIAL AND METHODS

The grazing experiments were conducted from June till the end of November 2003 at two areas, a young *Chamaecyparis* obtusa forest (YF) area (2 ha) and a native grassland (NG) area (3 ha) both in University of Miyazaki, southern Kyushu. In YF area, 176 species of herbs, vines, shrubs and trees besides grasses such as *Miscanthus sinensis* were observed[12] while in the NG area, *M. sinensis, Imperata cylindrica, Eremochloa ophiruiodes, Hermarthria sibirica* and *Pleioblastus sinensis* were mainly observed[2]. Three Japanese Black cows were initially set-stocked in the YF area and then in the NG area. The observation of behavior was conducted with 3 animals for 7 days of the experimental period. Behavior was recorded as grazing (specified as *M. sinensis, P.simonii* and other plants), rumination, resting and other categories with postures of lying and standing at 2 min. interval for 24 hr.

A Global Positioning System (GPS) apparatus[4] was attached on the neck of an animal to monitor the distance traced. It was set to record every 4 min. on the day of the behavioral observation.

Rumen fluid was collected twice in the YF and NG areas respectively, during early set-stock (8/03 in YF and 10/09 in NG) and during late set-stock(9/29 in YF and 11/22 in NG). The sample was collected through rumen cannula one hour after the end of the morning grazing and filtered with double cheese-cloth for the measurement of fermentation profile and bacterial and protozoal compositions. The portion of rumen sample was fixed with methyl green formalin saline solution to determine the number and compositional percentage of protozoa. The bacterial number was also counted according to the gram staining and shapes, after a fixation of 20% formalin saline solution[13]. The rumen samples were also immediately subjected to measurement of pH and kept at −20℃ until appropriate analysis. The concentrations of volatile fatty acids (VFA) and anmonium nitrogen ($NH_3$-N) were determined by high-pressure liquid chromatograph (Shimadzu, L C-10) equipped with Shim-pack SCR-102H (8 mmI.D *300mmL) and by a Kjeldahl system[1] respectively.

The behavioral data was analyzed by the Statistical Analysis Systems Institute software package[16].

## RESULTS AND DISCUSSION

Behavioral changes in grazing and lying are represented in Table 1. Total time spent grazing varied significantly over the days. It was significantly higher ($p<0.05$) on 9/28 and 11/21 when compared to that on 6/13, 7/12 and 8/02. The rate of grazing *M. sinensis* by percentage of total grazing time increased gradually from 14.8% to 30.0% during the first three observations in YF. After a drop in grazing time on 9/10, it increased ($p<0.05$) again to 32.8% on 9/

28. It was found to be the highest (63.4%, $p<0.05$) in NG on 11/21. The rate of grazing *P. simonii* by percentage of total grazing time was highest ($p<0.05$) on 6/13 among all the days. It gradually decreased ($p<0.05$) over the days in both YF and NG. The rate of grazing other plants was significantly higher ($p<0.05$) on 9/10 and 10/08 compared to the other days. The rate of lying posture by percentage of 24 hour was significantly higher ($p<0.05$) on 11/21 when compared to that on 9/10, 9/28 and 10/08.

Behavioral changes in resting and rumination over the days in YF and NG areas are shown in Table 2. No significant changes in total resting time and resting in lying posture (LRE) were found throughout the experimental period. While, resting in standing posture (SRE) was significantly highest ($p<0.05$) on 9/10 when compared to all the other days. Total rumination time and rumination in standing posture (SRU) did not show any significant changes over the days. Rumination in lying posture (LRU) was similar over the days in YF, and was found to be significantly highest ($p<0.05$) on 11/21 when compared to all the other days.

Correlation coefficients between different categories of behavior are shown in Table 3. Total grazing time was positively correlated with the rate of grazing *M. sinensis* ($r=0.436$, $p<0.05$) and negatively with grazing rate of *P. simonii* ($r=-0.676$, $p<0.001$). Grazing rate of *M. sinensis* was negatively correlated with the rates of grazing *P. simonii* ($r=-0.448$, $p<0.005$) and other plants ($r=-0.933$, $p<0.001$), and positively correlated with lying ($p<0.05$). Grazing rate of other plants was negatively correlated with time spent rumination and the rate of lying (r=

**Table 1. Behavioral changes of grazing and lying of Japanese Black cattle in young forest (YF) and native grassland (NG) areas**

| Date | Area | Behavior (average±SD) | | | | |
|---|---|---|---|---|---|---|
| | | Grazing | | | | |
| | | Total (min.) | *M. sinensis* (% of grazing time) | *P. simonii* (% of grazing time) | Other plants (% of grazing time) | Lying (% of 24 Hr) |
| 6/13 | YF | $406.7\pm41.6^{f}$ | $14.8\pm9.8^{c}$ | $19.1\pm5.9^{a}$ | $66.0\pm4.4^{cd}$ | $41.5\pm1.2^{ab}$ |
| 7/12 | YF | $503.3\pm115.0^{cdef}$ | $17.9\pm4.4^{c}$ | $9.3\pm3.0^{b}$ | $72.8\pm6.1^{bc}$ | $41.5\pm3.4^{ab}$ |
| 8/02 | YF | $436.0\pm52.6^{def}$ | $30.0\pm4.9^{b}$ | $9.6\pm2.5^{b}$ | $60.3\pm2.5^{de}$ | $41.6\pm5.9^{ab}$ |
| 9/10 | YF | $532.0\pm57.6^{bcd}$ | $12.3\pm3.6^{c}$ | $7.1\pm2.3^{bc}$ | $80.6\pm4.2^{ab}$ | $31.0\pm0.8^{c}$ |
| 9/28 | YF | $700.0\pm56.4^{a}$ | $32.8\pm6.9^{b}$ | $2.8\pm2.2^{cd}$ | $64.3\pm9.2^{d}$ | $37.7\pm9.1^{bc}$ |
| 10/08 | NG | $560.0\pm29.1^{bc}$ | $10.3\pm4.0^{c}$ | $1.5\pm1.7^{d}$ | $88.2\pm3.9^{a}$ | $39.4\pm2.2^{bc}$ |
| 11/21 | NG | $626.0\pm19.1^{ab}$ | $63.4\pm0.9^{a}$ | $0.5\pm0.2^{d}$ | $36.1\pm1.0^{f}$ | $48.9\pm5.6^{a}$ |
| All | | $537.7\pm109.8$ | $25.9\pm18.3$ | $7.1\pm6.6$ | $66.9\pm16.4$ | $40.2\pm6.5$ |
| P-value of ANOVA | | | | | | |
| DATE | | 0.0004 | <0.0001 | <0.0001 | <0.0001 | 0.022 |

abcdef Different superscripts in the same column are significantly different.

**Table 2. Behavioral changes of resting and rumination of Japanese Black cattle in young forest (YF) and native grassland (NG) areas**

| Date | Area | Behavioural category* (min/24 hr, average±SD) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Resting | | | Rumination | | |
| | | Total | SRE | LRE | Total | SRU | LRU |
| 6/13 | YF | $413.3\pm106.8$ | $146.7\pm51.5^{b}$ | $266.7\pm63.1$ | $426.0\pm98.8$ | $108.0\pm42.1$ | $318.0\pm64.2^{bc}$ |
| 7/12 | YF | $456.7\pm176.7$ | $142.0\pm46.5^{b}$ | $314.7\pm13.3$ | $326.7\pm103.1$ | $60.7\pm12.1$ | $266.0\pm91.1^{bc}$ |
| 8/02 | YF | $472.7\pm92.1$ | $171.3\pm21.4^{b}$ | $301.3\pm113.1$ | $380.0\pm74.1$ | $86.0\pm44.2$ | $294.0\pm30.2^{bc}$ |
| 9/10 | YF | $448.0\pm58.4$ | $240.7\pm14.2^{a}$ | $207.3\pm49.2$ | $342.7\pm52.8$ | $114.0\pm15.6$ | $228.7\pm38.1^{c}$ |
| 9/28 | YF | $320.0\pm88.4$ | $56.0\pm15.9^{c}$ | $264.0\pm104.1$ | $354.7\pm51.6$ | $81.3\pm74.9$ | $273.3\pm26.4^{bc}$ |
| 10/08 | NG | $262.7\pm98.8$ | $47.3\pm9.9^{c}$ | $215.3\pm89.6$ | $407.3\pm88.9$ | $62.0\pm27.8$ | $345.3\pm65.1^{b}$ |
| 11/21 | NG | $270.7\pm90.1$ | $20.0\pm5.3^{c}$ | $250.7\pm95.2$ | $501.3\pm76.1$ | $50.7\pm65.3$ | $450.7\pm37.1^{a}$ |
| All | | $377.7\pm124.3$ | $117.7\pm79.0$ | $260.0\pm88.6$ | $391.2\pm88.0$ | $80.4\pm44.8$ | $310.9\pm82.4$ |
| P-value of ANOVA | | | | | | | |
| DATE | | 0.111 | <0.0001 | 0.780 | 0.199 | 0.561 | 0.005 |

abc Different superscripts in the same column are significantly different.
*SRE: Resting in the standing posture; LRE: Resting in the lying posture;
SRU: Rumination in the standing posture; LRU: Rumination in the lying posture

−0.438 and r=−0.513 respectively. $p<0.05$).

Table 4 shows the distance covered by Japanese Black cattle in YF and NG areas by measured GPS. Distance covered by cattle was ranged from 5006.1 m on 8/02 to 6878.6 m on 9/10. In YF, it was negatively correlated with the rate of grazing *M. sinensis* and lying (r=−0.600 and r=−0.592 respectively, $p<0.05$). Longer distance was accompanied with smaller grazing rate of *M. sinensis* and shorter lying time.

The rate of grazing *M. sinensis* did not differ much in YF area while it increased in NG with availability of *M. sinensis*. And also it affected rumination time and the rate spent lying. It is considered that the availability of *M. sinensis* increased while of other plant species decreased rumination time due to the higher crude fiber content and lower digestibility in *M. sinensis* than in others[6,11]. Hasegawa and Hidari[5] have reported changes in grazing behavior of cattle with availability and quality of herbage. They observed that grazing time varied monthly and that low availability and quality of pasture prolonged grazing time. Suzuki *et. al.*[19] observed that greater herbage mass or lower nutritive value brought about longer rumination time and that smaller herbage mass brought longer grazing time.

As shown in Table 5, pH of the rumen fluid averaged 7 both in YF and NG. In YF, total VFA was 7.6 mmol/dl during both early and late set-stock periods. Among the individual VFA's, acetic acid decreased from 69.2% to 66.5% and propionic acid increased from 11.3% to 12.6% according to the stocking period. Again, while iso-butyric acid increased from 9.0% to 11.2%, n-butyric acid decreased from 10.4% to 9.7%. Thus ratio of acetic acid/propionic acid ratio was 6.10 and 5.30 at early and late set-stock respectively.

In NG, total VFA increased to 8 mmol/dl during early set-stock while it decreased to 7.4 mmol/dl during late set-stock. Though acetic acid increased to 70.9% after set-stock in the NG, it decreased to 68.4% during late set-stock. Propionic acid also increased to 13.3% when initially set-stocked in NG but decreased to 11.7% during late set-stock. Alternatively iso-butyric acid decreased during early set-stock to 8.2% and then increased to 12.3%. Also, n-butyric acid decreased initially to 7.5% and increased only marginally to 7.5%. A ratio of acetic acid/propionic acid ratio also increased slightly from 5.3 to 5.8 from early set-stock to late set-stock. No shifts in total VFA values were seen in YF while shifts in individual VFA's were noticed.

An increased percentage of acetic acid is the characteristic of plant cell wall fermenta-

Table 3. Correlation coefficients between different categories of behavior

| Behavioral categories | Grazing (min) | *M. sinensis* (% of grazing time) | *P. simonii* (% of grazing time) | Other plants (% of grazing time) | Rumination (min) | Lying (%) |
|---|---|---|---|---|---|---|
| Grazing (min) | - | 0.436* | -0.676*** | -0.214 | 0.295 | -0.186 |
| *M. sinensis* (% of grazing time) | - | - | -0.448* | -0.933*** | 0.395 | 0.503* |
| *P. simonii* (% of grazing time) | - | - | - | 0.097 | -0.004 | -0.118 |
| Other plants (% of grazing time) | - | - | - | - | -0.438* | -0.513* |
| Rumination (min) | - | - | - | - | - | 0.050 |
| Lying (%) | - | - | - | - | - | - |

*$p<0.05$, ***$p<0.001$

Table 4. Distance covered by Japanese Black cattle in young forest (YF) and native grassland (NG) areas measured by GPS

| Date | Distance covered |
|---|---|
| 6/13 (YF) | 6001.3 |
| 7/12 (YF) | Error |
| 8/02 (YF) | 5006.1 |
| 9/10 (YF) | 6878.6 |
| 9/28 (YF) | 5963.5 |
| 10/08 (NG) | 5341.9 |
| 11/21 (NG) | 6238.5 |

tion while higher percentage of propionate represents soluble carbohydrate fermentation. Previous work[15] has noted shifts in molar proportions of VFA in grazing ruminants between wet and dry seasons and growing and dormant seasons. HASEGAWA and HIDARI[5] observed that seasonal changes in chemical composition of herbage and dry matter intake influenced the differences in molar proportions of VFA. In this study, individual VFA could be contributed to the difference in the vegetation of the two areas.

In YF, $NH_3$-N concentration in the rumen increased from early set-stock 6.4 mg/dl to 7.2 mg/dl during late set-stock. With the cattle set-stocked in NG, $NH_3$-N decreased to 4.5 mg/dl during early set-stock and further decreased to 4.3 mg/dl during late set-stock. In this study $NH_3$-N concentrations in YF exceeded the optimal level of 5 mg/dl reported by SATTER and SLYTER[17] for efficient microbial growth. Also the $NH_3$-N concentrations both in YF and NG well exceeded the 1 to 2 mg/dl concentration proposed by PETERSON[14] for optimal fiber digestion. An increase in dietary nitrogen can increase the amount of rumen $NH_3$-N and branched chain VFA such as iso-butyric acid, as these are products of amino acid deaminat ion. This was observed to occur in YF during late set-stock. PLAYNE and KENNEDY[15] observed a two-fold increase in the rumen $NH_3$-N concentrations during wet versus dry seasons of the year and attributed it to high dietary nitrogen levels.

Table 6 shows changes of rumen protozoa and bacteria of Japanese Black cattle in YF and NG areas. In YF, the total rumen protozoa were $2.0 \times 10^6$ during early set-stock and decreased to $3.0 \times 10^5$ during late set-stock.

Table 5. Change of rumen fluid parameters of Japanese Black cattle in young forest (YF) and native grassland (NG) areas

| Item | Area and Date | | | |
|---|---|---|---|---|
| | YF | | NG | |
| | 8/03 | 9/29 | 10/09 | 11/22 |
| pH | 7.2 | 6.9 | 7.0 | 7.2 |
| Total VFA, mmol/dl | 7.6 | 7.6 | 8.0 | 7.4 |
| Acetic acid, molar% | 69.20 | 66.52 | 70.98 | 68.41 |
| Propionic acid, molar% | 11.33 | 12.55 | 13.30 | 11.79 |
| Iso-butyric acid, molar% | 9.04 | 11.21 | 8.24 | 12.29 |
| N-butyric acid, molar% | 10.43 | 9.73 | 7.48 | 7.52 |
| Acetic acid/propionic acid | 6.10 | 5.30 | 5.30 | 5.80 |
| $NH_3$-N, mg/dl | 6.4 | 7.2 | 4.5 | 4.3 |

Table 6. Changes of rumen protozoa and bacteria of Japanese Black Cattle in young forest (YF) and native grassland (NG) areas

| | Area and Date | | | |
|---|---|---|---|---|
| | YF | | NG | |
| | 8/03 | 9/29 | 10/09 | 11/22 |
| Protozoa | | | | |
| Total, cells/ml | $2.0 \times 10^6$ | $3.0 \times 10^5$ | $1.0 \times 10^6$ | $1.0 \times 10^6$ |
| *Entodinium*, % | 75.01 | 73.60 | 49.07 | 66.10 |
| *Diplodinium*, % | 13.05 | 1.01 | 14.81 | 15.25 |
| *Ophryoscolex*, % | 1.35 | 0.29 | 0.93 | 9.32 |
| *Epidinium*, % | 2.85 | 0.70 | 5.56 | nd* |
| *Polyplastron*, % | 3.26 | nd | 0.93 | nd |
| *Dasytrichia*, % | 0.41 | 12.78 | 9.26 | 9.32 |
| *Isotrichia*, % | 4.08 | 11.62 | 19.44 | nd |
| Bacteria | | | | |
| Total, cells/ml | $3.7 \times 10^7$ | $8.2 \times 10^8$ | $1.4 \times 10^7$ | $2.3 \times 10^7$ |
| cocci(+), % | 38.60 | 47.30 | 5.74 | 38.20 |
| cocci(-), % | 37.00 | 38.30 | 40.40 | 46.22 |
| cocci pair(-), % | 3.68 | 5.00 | 13.40 | 4.24 |
| rod round(-), % | 1.11 | nd | 14.39 | nd |
| Others, % | 19.68 | 10.4 | 26.07 | 11.22 |

*nd;not ditected.

The genus *Entodinium* occupied 75.0% and 73.6% of the total percent composition during early set-stock and late set-stock, respectively. During early set-stock, the genus *Diplodinium* occupied about 13.1% of the total percent composition. During late set-stock, *Dasytrichia* and *Isotrichia* increased to 12.8% and 11.6% respectively.

In NG, the total protozoa were found to be $1.0 \times 10^6$ during both early set-stock and late set-stock. During early set-stock the percent composition of *Entodinium* decreased to 49.1 % and that of *Dasytrichia* to 9.3%. While those of *Diplodinium* and *Isotrichia* increased to 14.8% and 19.4%, respectively. During late set-stock in the grassland area, *Entodinium* increased to 66.1% and a slight increase in the percentage of *Diplodinium* to 15.3% was seen. While *Isotrichia* was not detected during late set-stock, *Ophryoscolex* and *Dasytrichia* occupied about 9.3% of the total composition.

Total number and genus composition can be affected by diet type and feeding frequency[20]. In this study, the total number of protozoa decreased by changing from the YF area to the NG area. Though the types of protozoa did not differ, differences in percentages of the types were observed. Entodiniomophid protozoa are generally prevalent with large amounts of hemicellulose and cellulose[3] while *Isotrichia* protozoa are prevalent with soluble sugars[21]. In our study, the percentage of *Entodinium* and *Diplodinium* was high during early set-stock in the YF area and late set-stock in the grassland area, while *Dasytrichia* and *Isotrichia* were found in high percentages during late set-stock in the YF area and early set-stock in the NG area. These differences could be due to the changes in forage quality during the particular periods.

The total rumen bacteria in YF increased from $3.7 \times 10^7$ to $8.3 \times 10^8$ from early set-stock to the late set-stock. Percentage of gram positive cocci increased from 38.6% to 47.3%, from early set-stock to late set-stock. Gram negative cocci remained at 37% of the total during early set-stock and 38% during late set-stock. In NG, the total rumen bacteria decreased during early set-stock to $1.4 \times 10^7$ and increased again to $2.3 \times 10^7$ during late set-stock. Gram positive cocci increased from 5.7% to 38.2% from early set-stock to late set-stock gram negative cocci increased from 40.2% to 46.2%. Gram negative cocci pair and gram negative rod round were found to be 13.4% and 14.4% during early set-stock but gram negative cocci pair decreased to 4.2% and rod round-was not detected during late set-stock. Though gram negative cocci were present in large nu mbers, gram positive cocci also occupied a high percentage of the total, except during early set-stock in the grassland pasture.

This study shows that the cattle were able to adapt well in both the young forest area and native grassland area, providing the needed nutrients.

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# 幼齢造林地および野草地放牧黒毛和種牛の行動とルーメン内性状の特性

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## 要　　約

幼齢ヒノキ造林地（YF 区，2003年6－9月）および野草地（NG 区，2003年10・11月）に放牧された黒毛和種雌牛の行動とルーメン内性状の特性を明らかにするために、24時間行動観察とGPSによる移動距離測定を各月1回，ルーメン液採取を各区2回行った。採食行動時間は平均537.7±109.8分／日で、*Miscanthus sinensis* 採食割合と正（$r=0.436$，$p<0.05$）、*Pleioblastus simonii* 採食割合と負（$r=-0.676$，$p<0.001$）の有意な相関を示した．*M. sinensis* 採食割合は、*P. simonii* およびその他の植物採食割合と負（$p<0.001$）、横臥姿勢割合と正（$p<0.05$）の相関を示した。放牧期間中の移動距離は5001－6879m であった。ルーメン液中総 VFA 濃度に大きな変動はみられなかったが、個々の脂肪酸割合には牧区と時期によって変動に違いがみられた。$NH_3$-N 濃度は YF 区が NG 区よりも高かった．総プロトゾア数／ml は YF で放牧初期 $2.0 \times 10^6$ から放牧後期 $3.0 \times 10^5$ に減少し、NG では変化は示さず $1.0 \times 10^6$ で、両区とも *Entodinium* 割合が最も高くかった。総バクテリア数／ml は $1.4 \times 10^7 - 8.2 \times 10^8$ で、cocci(＋)と cocco(-)の割合が高かった。この研究において、牛は幼齢造林地と野草地放牧に、行動を変化させ多様な植物を選択することで適応する能力があることが示された。

キーワード：採食行動、黒毛和種牛、幼齢造林地、野草地、ルーメン内性状

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