原　著

# 牛尿浸漬処理による稲わらの栄養価向上

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# Improvement of the Voluntary Intake and Digestibility of Rice Straw by Soaking in Cattle Urine

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## Summary

Rice straw was soaked in fresh and condensed cattle urine, and the *in situ* and *in vivo* digestibility of the treated rice straw was compared with untreated rice straw by sheep. Soaking rice straw in cattle urine resulted in increases in crude protein content and decreases in neutral detergent fiber, acid detergent lignin, and hemicellulose content. The treatment improved *in vivo* and *in situ* digestibility and voluntary dry matter intake of sheep. *In situ* digestibility of untreated and treated rice straw incubated in the rumen of sheep fed urine treated rice straw was higher than those in the rumen of sheep fed untreated rice straw. The treatment with condensed urine was more effective in changing chemical composition, *in situ* and *in vivo* digestibility. These results indicate that the rumen environment of sheep was enhanced in its degrading ability of rice straw by feeding of urine treated rice straw, in addition to the chemical effect of the treatment on the digestibility of rice straw.

## 要　約

稲わらを牛尿および2倍濃縮した牛尿に一晩浸漬し，乾燥後第一胃フィステルを装着した3頭のめん羊に給与して消化試験を実施した．試験は無処理稲わら，新鮮牛尿処理稲わらおよび濃縮尿処理稲わらの3種を試験飼料とした3×3ラテン方格法により実施し，第一胃における*in situ*消化率と*in vivo*消化率を同時に測定した．稲わらを牛尿に浸漬することにより，粗蛋白質含量は増加し，中性デタージェント繊維，酸性デタージェントリグニンおよびヘミセルロース含量は減少した．牛尿浸漬処理により*in vivo*消化率および*in situ*消化率は向上し，自由乾物摂取量は増加した．牛尿浸漬処理をした稲わらを給与しためん羊の第一胃内における処理稲わらと無処理の稲わらの*in situ*消化率は無処理の稲わらを給与しためん羊の第一胃内よりも高かった．濃縮尿に浸漬することによる稲わらの化学組成，*in vivo*および*in situ*消化率に対する効果は新鮮尿の効果より高くなった．以上の結果から，稲わらの牛尿浸漬処理は自由乾物摂取量と消化率を向上させるが，その効果は直接消化率を向上させる効果に加え，それを摂取しためん羊の第一胃内における分解性を向上させる効果も加算されるものと考えられた．

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## INTRODUCTION

Rice straw is characterized by low voluntary intake, low digestibility, and low protein content. Many attempts have been made to improve the nutritive value of cereal straws by mechanical, chemical, and biological processing (KLOPFENSTEIN 1978; ITOH 1983; OKAMOTO and ABE 1989; YAMAKAWA *et al.* 1992). The traditional BECKMANN method with sodium hydroxide solution has the disadvantages of high usage of water and alkali, loss of nutrients into the water, and polluting water-courses (OWEN 1979). The industrial "dry method" has been developed for overcoming these disadvantages but the "dry method" is not suitable for on-farm processing. Ammonia treatment of straw can improve nitrogen content in addition to the effect of alkali treatment (ITOH 1983). However, ammonia treatment requires airtight coverage and a longer period for treatment, and involves hazardous chemicals. The treatment of straw with urea is a kind of ammonia treatment because urease in the straw generates ammonia from urea. SAADULLAH *et al.* (1980) used sheep urine as a urea source for ammonia treatment of rice straw, and reported improved dry matter and crude fiber digestibility after 20 days reaction period in a covered stack.

This study attempted to improve the nutritive value of rice straw by soaking in cattle urine overnight. The objective of the study was to determine the effect of this simple and time saving treatment with a natural urea source on the nutritive value of rice straw.

## MATERIALS AND METHODS

Rice straw was purchased from a local rice farmer in Hokkaido. Cattle urine was collected directly with a bucket from lactating dairy cows in the dairy barn of Rakuno Gakuen University. A small amount of sulfuric acid was added to the collected urine and then used in two ways; fresh urine and condensed to half weight. The urine was concentrated in shallow plates at 60 ℃ in a draft. Approximately 3 kg of rice straw was soaked in about 50 litters of the fresh (URS) or condensed urine (2URS) in a large bucket for about 14 hours, drained, and dried at 60℃ for 24 hours. Untreated rice straw (RS), rice straw treated with fresh urine and rice straw treated with 2 times condensed urine were used in the experiments after cutting to 3 to 5 cm length.

Digestion experiments were done in two ways; *in situ* and *in vivo* trials. For *in situ* and *in vivo* digestion experiments, three adult castrated male Suffolk sheep with rumen fistula were used in a 3×3 Latin square design. The body weights of the sheep were between 46 and 52 kg during the experiment. The sheep were kept in individual metabolic cages and allocated to one of the three straw diets, RS, URS, or 2URS, according to the experimental design. The straw diets were cut to about 3 cm length and offered *ad libitum* at 10:00 and 17:00 h daily. Each experimental period lasted 15 days, 10 days for adaptation and 5 days for collection. Feed intake was recorded every day and samples of feed, leftover of feed, feces, and urine were collected during the collection period. All samples except urine were dried at 60℃ and were ground through a WILEY mill with a 1 mm screen for analysis. The animals were treated in accordance with guidelines outlined by the Rakuno Gakuen University Animal Care and Use Committee. The committee approved this study.

For *in situ* experiments, all sheep had three nylon bags with 10 g ground RS sample inserted into the ventral sac of the rumen before feeding on the third day of the collection period. The sheep fed URS or 2URS also had nylon bags with ground URS or 2URS sample inserted into the rumen, respectively. The nylon bag was 80×150 mm and with 50 $\mu$m pore size. These nylon bags were immersed into water for 20 minutes before insertion into the rumen. The nylon bags were removed from the rumen after 12, 24, and 48 hours incubation and rinsed in cold tap water until the rinsing water was clear. After drying at 60℃, samples in the *in situ* bag were prepared for chemical analysis.

The dry matter (DM), organic matter (OM), crude protein (CP), and ether extracts (EE) were determined according to AOAC (1990). Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were determined by the method of GOERING and VAN SOEST (1970). In accordance with the method of OKAMOTO and HIROSE (1972), hemicellulose (HCEL) and cellulose (CEL) were determined as the difference between NDF and ADF and between ADF and ADL, respectively. Combustion energy was measured by an automated bomb calorimeter (Shimadzu CA-3, Kyoto, Japan).

The data from the *in situ* and *in vivo* trials was subjected to analysis of variance for 3×3 Latin square. TUKEY's test was used to identify differences ($p<0.05$) between means (STEEL and TORRIE 1980).

## RESULTS AND DISCUSSION

The chemical composition of untreated and urine treated rice straw is presented in Table 1. The rice straw used in the experiment was poor quality with very low CP and high fiber contents. Soaking the rice straw in fresh cattle urine increased CP and EE contents and decreased OM, NDF, ADF, and ADL contents. The decrease of OM was due to the increase of crude ash, and the decrease in fiber fractions was mainly due to the decrease in the HCEL fraction. The changes in chemical composition were further enhanced by soaking rice straw in condensed cattle urine. However, CEL content did not change significantly. The pH of the urine samples in the university herd fed similar TMR to the present herd ranged from 8. 14 to 8. 63. The urea nitrogen and ammonia nitrogen content of dairy cattle urine was 5305±1559 mg/kg and 140. 8±38. 0

Table 1 Chemical composition of rice straw, urine treated rice straw, and rice straw treated with condensed urine (% in DM)

| | RS | URS | 2URS |
|---|---|---|---|
| Organic matter | 84. 9 | 80. 1 | 76. 7 |
| Crude protein | 4. 3 | 7. 2 | 10. 2 |
| Ether extract | 1. 3 | 1. 5 | 2. 8 |
| Neutral detergent fiber | 71. 1 | 62. 4 | 57. 7 |
| Acid detergent fiber | 50. 3 | 48. 8 | 47. 7 |
| Acid detergent lignin | 7. 0 | 4. 7 | 4. 2 |
| Hemicellulose | 20. 8 | 14. 0 | 10. 0 |
| Cellulose | 43. 2 | 44. 1 | 43. 5 |
| Combustion energy ( kJ/g DM ) | 15. 5 | 16. 3 | 16. 3 |

RS: Untreated rice straw; URS: urine treated rice straw; 2URS: rice straw treated with 2 times condensed urine.

mg/kg, respectively (OKAMOTO *et al.* 2001). It was supposed that the rice straw absorbed urea and ammonia nitrogen during the soaking treatment. The water intake of sheep fed URS and 2URS increased from 3. 0 kg/day to 3. 5 and 4. 6 kg/day, respectively.

The NDF and ADL content decreased in the treated rice straw, and resulted in a decrease in HCEL and lignin content. These results were similar to the effect of alkali treatment with chemicals such as sodium hydroxide and ammonia. Hemicellulose and lignin together form the encrusting material of the secondary wall thickening. Alkali cleaves ester bonds, generally breaking lignin-carbohydrate bonds and thereby causing increasing solubility in neutral detergent solution (VAN SOEST 1982). Cattle urine might have a similar effect to alkali in cleaving lignin-carbohydrate bonds.

Voluntary DM intake of sheep fed RS, URS, and 2URS is shown in Table 2. Table 2 also illustrates *in vivo* digestibility of chemical components. Voluntary DM intake of RS was extremely low. The DM intake increased by more than 50% after the urine and condensed urine treatments. The sheep appeared to prefer URS and 2URS, and the palatability of the treated straw was better than RS.

The digestibility of DM, OM, CP, and energy increased significantly following fresh urine treatment, and the digestibility of fiber fractions, such as ADF, HCEL, and CEL, increased significantly following treatment with condensed urine. The digestible energy content of RS, URS, and 2URS was calculated to be 6. 69, 9. 33, and 10. 58 kJ/g DM, respectively. Nitrogen retention per day was improved from -2. 95 g to +0. 21 g for URS and to +1. 99 g for 2URS ($p<0.01$).

Table 2 Voluntary dry matter consumption and digestibility of rice straw, urine treated rice straw, and rice straw treated with condensed urine (%)

| | RS | URS | 2URS | SEM |
|---|---|---|---|---|
| Dry matter intake (g/day) | 386. 3$^{a}$ | 581. 9$^{b}$ | 628. 3$^{b}$ | 12. 6 |
| Digestibility | | | | |
| Dry matter | 31. 1$^{a}$ | 46. 3$^{b}$ | 58. 6$^{b}$ | 1. 64 |
| Organic matter | 31. 8$^{a}$ | 46. 5$^{b}$ | 57. 4$^{b}$ | 1. 64 |
| Crude protein | 43. 2$^{a}$ | 57. 3$^{b}$ | 65. 6$^{b}$ | 1. 15 |
| Ether extract | 46. 8 | 60. 0 | 81. 9 | 5. 57 |
| Neutral detergent fiber | 33. 2 | 39. 5 | 48. 6 | 1. 84 |
| Acid detergent fiber | 24. 1$^{a}$ | 28. 6$^{ab}$ | 42. 4$^{b}$ | 1. 83 |
| Hemicellulose | 40. 1$^{a}$ | 51. 6$^{a}$ | 64. 2$^{b}$ | 1. 44 |
| Cellulose | 28. 2$^{a}$ | 33. 7$^{ab}$ | 45. 1$^{b}$ | 1. 93 |
| Energy | 42. 9$^{a}$ | 57. 0$^{b}$ | 65. 0$^{c}$ | 0. 58 |
| Digestible energy (kJ/g) | 6. 69$^{a}$ | 9. 33$^{b}$ | 10. 58$^{c}$ | 0. 08 |
| Nitrogen retention (g/day) | -2. 95$^{a}$ | 0. 21$^{b}$ | 1. 99$^{c}$ | 0. 02 |

RS: Untreated rice straw; URS: urine treated rice straw; 2URS: rice straw treated with 2 times condensed urine.

$^{a,b,c}$ Values on the same row with different superscripts show significant differences ($p<0.05$).

SEM: Standard error of the treatment mean.

Table 3 *In situ* digestibility of rice straw, rice straw treated with urine, and rice straw treated with condensed urine in the rumen of sheep fed rice straw, rice straw treated with urine, and rice straw treated with condensed urine (%)

| Incubation hour | RS/RS* | RS/URS | RS/2URS | SEM | URS/URS | 2URS/2URS | SEM |
|---|---|---|---|---|---|---|---|
| Dry matter | | | | | | | |
| 12 | 13.0$^{x}$ | 15.5 | 19.3 | 1.55 | 29.8$^{y}$ | 30.9$^{y}$ | 2.00 |
| 24 | 23.7$^{ax}$ | 37.4$^{ab}$ | 48.1$^{b}$ | 1.99 | 50.5$^{y}$ | 64.7$^{z}$ | 0.47 |
| 48 | 32.2$^{ax}$ | 42.7$^{ab}$ | 53.8$^{b}$ | 1.93 | 60.9$^{y}$ | 67.6$^{z}$ | 0.51 |
| Hemicellulose | | | | | | | |
| 12 | 19.0$^{x}$ | 21.1 | 24.3 | 1.43 | 32.0$^{y}$ | 35.0$^{z}$ | 0.33 |
| 24 | 28.0$^{ax}$ | 39.4$^{ab}$ | 48.3$^{b}$ | 1.74 | 49.3$^{y}$ | 61.1$^{z}$ | 0.47 |
| 48 | 35.1$^{ax}$ | 43.9$^{ab}$ | 53.1$^{b}$ | 1.86 | 57.4$^{y}$ | 63.4$^{z}$ | 0.10 |
| Cellulose | | | | | | | |
| 12 | 10.2$^{x}$ | 12.6 | 16.1 | 1.52 | 22.5$^{y}$ | 24.3$^{z}$ | 0.80 |
| 24 | 20.1$^{ax}$ | 32.9$^{ab}$ | 42.8$^{b}$ | 2.47 | 42.2$^{y}$ | 55.2$^{z}$ | 0.76 |
| 48 | 28.0$^{ax}$ | 37.8$^{ab}$ | 48.0$^{b}$ | 2.07 | 51.4$^{y}$ | 57.8$^{z}$ | 0.11 |

RS: Untreated rice straw; URS: Urine treated rice straw; 2URS: Rice straw treated with 2 times condensed urine.

*The numerator is the substrate and the denominator indicates the rumen environment (e.g. RS/URS: RS incubated in the rumen of sheep fed URS).

SEM: Standard error of the treatment mean.

$^{a,b}$ :Values with different superscripts among RS/RS, RS/URS, and RS/2URS show significant differences ($P<0.05$).

$^{x,y,z}$: Values with different superscripts among RS/RS, URS/URS, and 2URS/2URS show significant differences ($P<0.05$).

Table 3 shows *in situ* DM, HCEL, and CEL digestibility of RS in the rumen of sheep fed RS, URS, or 2URS. The table also illustrates *in situ* DM, HCEL, and CEL digestibility of URS or 2URS in the rumen of sheep fed URS and 2URS, respectively. The *in situ* digestibility of RS tended to improve in the rumen of sheep fed URS and improved significantly in the rumen of sheep fed 2URS after 24-hour incubation. Most values of the *in situ* digestibility of DM and the fiber fraction of URS and 2URS in the rumen of sheep fed URS and 2URS were significantly higher than RS digestibility in the rumen of sheep fed RS.

The urine treatment decreased NDF and ADL content, and improved *in situ* and *in vivo* digestibility of the rice straw. This fact indicated the breaking hemicellulose-lignin bonds, and might lead to damage to the physical incrustation of the cell wall and might reduce some other factors limiting digestibility.

In the present study, *in situ* digestibility of RS was determined in the rumen of sheep fed RS, URS, or 2URS. This provided useful information of the rumen environmental effect on degradability of rice straw. The results indicated that the rumen environment of sheep fed urine treated rice straw had a greater ability to degrade rice straw. The microorganisms in the rumen may be encouraged to degrade rice straw by consuming treated straw. The urine treatment should provide more nitrogen and energy sources for microbial fermentation.

The total improvement of *in situ* digestibility of URS and 2URS in the rumen of sheep fed the corresponding treated straw consisted of an improvement in the rumen environment and from the direct effect of the treatments. The treatment effect might be estimated by subtracting the rumen effect from the total improvement. The treatment effect appeared in earlier stages of incubation than the rumen effect. This suggested that the urine treatment had an immediate effect on digestibility; however, the rumen effect required some lag before sufficient activation occurred.

Voluntary intake of rice straw by sheep was improved remarkably by the soaking treatment in the cattle urine. OKAMOTO and ABE (1990) and OKAMOTO and MIYAZAKI (1990) indicated that ammonia and steam treatment of rice straw improved voluntary intake not only by higher digestibility but also by accelerating particle size reduction and higher passage rate. The half-day urine treatment might have a similar effect to the ammonia treatment on chemical composition, digestibility and also voluntary consumption. Soaking rice straw in liquid urine results in quick absorption of urea and enhanced breaking lignin-carbohydrate bonds in the cell wall matrix. The combination enables shorter treatment period than the treatment with ammonia or other ammonia releasing compounds. The safety and time saving treatment with cattle urine is expected to be practical for improving nutritive value of rice straw.

In practice, collection of enough cattle urine without contamination of feces may be difficult. Urea solution may replace with cattle urine. Further research is required to confirm the effect of urea solution on the nutritive value of rice straw.

It was concluded that the soaking treatment of rice straw in cattle urine overnight improved DM intake and digestibility either through direct effects on chemical composition and digestibility or increased ability of degradation in the rumen.

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