EFFECT OF ADDITION OF INTACT OR ALKALIZED LUCERNE JUICE AT ENSILING ON THE NUTRITIVE VALUE OF RICE STRAW SILAGE

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Summary

This experiment was conducted to study the effect of addition of lucerne juice (LJ) obtained by mechanical extraction of freshly harvested crop on the nutritive value of rice straw silage. Rice straw (RS) was ensiled with intact, NaOH or NH_3 treated LJ at 3:7 ratio on fresh weight basis (LJ · RS, LJ · NaOH · RS and LJ · NH_3 · RS, respectively). Each alkali was mixed with fresh juice at a level of 4% of rice straw dry matter just before ensiling. Rice straw ensiled with water was prepared as the control (W · RS). In the digestion trial, goats were allocated in a 4 × 4 Latin-square design and fed the diet containing three parts of RS silage and one part of wheat bran (DM basis). For the goats receiving the control silage, urea was supplemented at feeding time so as to adjust the nitrogen intake except for goats on LJ · NH_3 · RS silage.

Crude protein content of RS silage was increased from 5.2 to 9.1% (DM basis) by the addition of intact LJ and to about 24% by NH_3 treated LJ. The control W · RS silage contained only trace amount of lactic acid and was dominated by acetic and butyric acid. The addition of intact LJ reduced butyric acid content and NH_3-N/TN of the silage whereas the addition of alkalized LJ increased those values and shifted to a butyrate type fermentation.

Nutrient digestibilities and nitrogen balance of goats were almost the same when they were fed W · RS and LJ · RS silage indicating the addition of intact LJ did not improve the nutritive value. The addition of alkalized LJ significantly increased the fiber digestibilities of RS silage and NH_3 treatment was more effective than NaOH treatment. Postprandial ruminal NH_3-N and blood urea nitrogen (BUN) concentrations were decreased by feeding LJ · NaOH · RS silage suggesting ruminal protein synthesis was enhanced along with the increase of energy supply for rumen microbes by the alkali treatment. The advantageous fiber digestibilities of LJ · NH_3 · RS silage compared with those of LJ · NaOH · RS silage might be attributable to a sufficient nitrogen supply for microbial fiber digestion in the rumen.

(**Key Words**: Alkali Treatment, Digestibility, Goat, Lucerne Juice, *Medicago sativa*, Rice Straw)

Introduction

Frequent cutting of forages at immature stage enables us to recover a maximum nutrient from the field. The crude protein content of such forages is usually greater than the level required by ruminants. These excessive protein can be separated and utilized through the process of green crop fractionation, which involves macerating the material, squeezing out forage juice and dividing the juice into leaf protein concentrate (LPC) and brown juice. Partial dejuicing of freshly harvested forage gives us a fibrous residue (pressed cake) without much DM loss which is normally associated with field wilting (Dumont and Boyce, 1976). Pressed cake is easily ensiled because of considerably high DM content and low buffering capacity (Ohshima and Oouchi, 1979). Thus, the process allows forages to be harvested and conserved at proper times independent of weather. Although pressed cake has lower protein and higher cell wall constituents than the material, it is utilized as a nutritious feedstuff as the original material by ruminants (Houseman and Connell, 1976; Ohshima et al., 1988, 1991). The LPC contains over 50% of protein in DM and plays as a highly available protein source for non-ruminant animals. However, preparation of LPC from forage juice, usually by heat coagulation and successive drying the coagulum, needs much energy consumption. Therefore, direct use of the juice as animal feed

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could substantially reduce the economy of the whole process (Beker et al., 1983). There have been many experiments on feeding pigs (Barber et al., 1979; Ohshima and Ueda, 1982) and rats (Hanczakowski, 1979). The Juice extracted from herbages of vegetative stage contains much soluble components besides protein. Therefore, the addition of it to low quality roughages at the time of ensiling is very effective in improving the chemical and nutritional qualities of the silage. And the alkalization of forage juice prior to the addition would improve the digestibility of fibrous components. To obtain forage juice, a facility is necessary. But once the facility is established, forage juice can easily be obtained by pressing herbage mechanically. The labour for the application of alkalized juice depends on the scale of silage making. Mechanical spraying demands very little labour.

Rice straw is a most abundant resource for ruminants in Asia but, unfortunately, low protein content and highly crystallized fiber of it limit its availability. When animals are fed such low quality roughages, nitrogen supplementation is effective for increasing digestibility, voluntary intake and successive animal performance (Campling et al., 1962; Smith et al., 1980). Moreover, chemical treatment of the roughage has been recently focused on because greater effects could be expected and it has been considered as a most applicable technique for practical use (Barreveld, 1989).

The present experiment was conducted to study the effect of addition of intact or alkalized lucerne juice at ensiling on improving the nutritive value and fermentation quality of rice straw silage.

Materials and Methods

Preparation of lucerne juice (LJ)

Second growth of lucerne (*Medicago sativa* L.) at early blooming stage was harvested on July 21st, 1988. The fresh crop was roughly disrupted with a herbage crusher (Nihon Sharyo Ltd.) and squeezed out LJ with a twin roll screw press (Stord Bartz Japan Ltd., TP-24-2.). The LJ, obtained 550 *l* by pressing 830 kg of the material, was stored in a bulk cooler at 4℃ until the next day when preparation of silage has been made.

Preparation of rice straw silage

Rice straw (RS) cut into 2 cm length was mixed with intact (fresh), NaOH or NH_3 treated (alkalized) LJ at 3 : 7 ratio on fresh weight basis and ensiled into polyethylene bag as to be 15 kg per bag. Those were referred to LJ · RS, LJ · NaOH · RS and LJ · NH_3 · RS, respectively. Each alkali was added at a level of 4% of RS dry matter by mixing its solution with fresh LJ just before ensiling. Rice straw ensiled with water was also prepared as the control (W · RS). All bags were preserved in a dark room at ambient temperature and opened one by one after about one year when animal trial was started.

Animal trial

Four Japanese pigmy castrated male goats weighing about 11.3 kg were individually housed in metabolism cages and were continuously fed 4 kinds of diets for 12 days each according to 4 × 4 Latin-square design. The whole duration of the animal trials was 48 days. Diets, each being offered at 2.5% of the body weight daily in dry matter basis, were composed of 3 : 1 mixture (DM basis) of RS silage and wheat bran so that the goats could receive enough protein to maintain their body weights. Feeds were offered in two equal meals at 09:00 and 17:00 hours. For the goats receiving the control silage, 1.5 g of urea supplying as an equivalent nitrogen as intact or NaOH treated LJ was supplemented at each feeding to investigate that the LJ could have efficacy on improving the nutritive value rather than non protein nitrogen addition. Two grams of sodium chloride and 10 g of dicalcium phosphate were fed with the morning meal. Water was freely accessible at all times. Feces and urine were collected for the last 5 days of each experimental period. All feces were collected, dried and composited as 5-day pooled samples. Urine was preserved with concentrated sulfuric acid and also composited. Rumen fluid was taken at 4 hours after morning meal by means of a stomach tube on a day during the collection period. Jugular blood sample was taken into heparinized tube at the same time.

Analyses

The toluene dry matter (Dewar and McDonald, 1961) and nitrogen content of silage were determined with fresh silage sample. Other chemical components of feeds and feces were determined using samples dried in a forced air oven at 60℃ for 48 hours. Analyses of nitrogen and ash were carried out according to AOAC (1984) procedure. Ash free neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined by the methods of Van Soest and Wine (1967) and Van Soest (1963), respectively. Starch hydrolysis by α-amylase (Abe and Horii, 1979) preceding neutral detergent digestion of wheat bran was made to facilitate filtering its residue. Hemicellulose and cellulose were considered as NDF minus ADF, and ADF minus ADL, respectively. Calory content was measured using a bomb calorimeter (Shimadzu, CA-3). Water soluble carbohydrate contents in LJ and RS were determined by the method of McDonald and Henderson (1964).

Chemical quality of silages was determined on cold water extract of each silage. Analyses of rumen fluid were carried out with the filtrates through double gauze. The pH value by a grass electrode pH meter (Horiba, F-12) and total concentrations of VFA and NH_3-N by steam distillation, were analyzed using these samples. Individual VFAs were gaschromatographycally determined on the distillates of the samples. Lactic acid in silages was determined according to the method of Barnett (1951). Blood urea nitrogen (BUN) concentration was measured with blood plasma using a commercial kit (Chugai Pharmaceutical, Ltd.). Data were subjected to analysis of variance and treatment means were compared using Duncan's multiple range test.

Results and Discussion

Dry matter, crude protein and water soluble carbohydrate (WSC) contents of intact LJ were 6.04, 2.02 and 0.71%, respectively (table 1). The crude protein content was about two times higher than, but WSC was as only one-fourth as that of ryegrass green juice (GJ) used in the previous study (Nishino et al., 1991). Generally, LJ is high in protein but low in WSC content compared with GJ while their contents are greatly influenced by fertilizer application (Cheeseman, 1977). Crude protein content of RS silage was increased from 5.2% to 9.1% (DM basis) by ensiling with intact LJ and to about 24% with NH_3 treated LJ (table 2). The fiber contents of W · RS silage were higher than those of RS ensiled with various LJ reflecting the composition of LJ. The addition of NaOH or NH_3 treated LJ significantly reduced hemicellulose content of the silages showing the solubility of hemicellulose was increased by the alkali treatment. Although it have been considered that NH_3 treatment did not affect ADF and cellulose contents of the treated roughage (Klopfenstein et al., 1972; Itoh, 1983), those contents of LJ · NH_3 · RS silage were higher than those of LJ · RS silage. These observations were ascribed to underestimation of sample dry matter determined with oven drying procedure. The loss of volatile materials during drying was probably high in LJ · NH_3 · RS silage because of the highest NH_3 and butyric acid contents of the silage (table 3).

Chemical quality of the silage is described in table 3. The control W · RS silage showed a trace of lactic acid content and the dominance of acetic and butyric acid contents. The addition of intact LJ improved chemical quality of RS silage with reduction of butyric acid content and NH_3-N/TN of the silage, however, it did not affect the lactic acid content and the pH value of RS silage. The WSC content of the silage material was increased from 3.84 to 4.91% of DM by the LJ addition,

TABLE 1. CHEMICAL COMPOSITION OF LUCERNE JUICE (LJ) AND RICE STRAW

Item	Intact LJ	LJ treated with NaOH	LJ treated with NH_3	Rice straw
pH	5.90	13.6	11.0	–
Dry matter[1)]	6.04	7.71	6.25	87.6
Crude protein[1)]	2.02	1.97	9.25	4.72
Ash[1)]	1.64	3.75	1.70	15.4
Water soluble carbohydrate[1)]	0.71	–	–	3.36

[1)] Percent of fresh matter.

TABLE 2. CHEMICAL COMPOSITION OF THE SILAGES

Item	W · RS[1]	LJ · RS	LJ·NaOH·RS	LJ·NaOH·RS
Crude protein (% DM)	5.15^{c}[2]	9.06^{b}	9.20^{b}	23.8^{a}
Ash (% DM)	17.9^{c}	19.8^{b}	21.6^{a}	20.2^{b}
NDF (% DM)	70.4^{a}	64.1^{b}	60.6^{c}	63.5^{b}
ADF (% DM)	43.0^{a}	38.4^{c}	39.3^{c}	41.3^{b}
ADL (% DM)	8.20^{a}	6.90^{b}	7.33^{b}	7.48^{b}
Hemicellulose (% DM)	27.4^{a}	25.7^{b}	21.3^{c}	22.2^{c}
Cellulose (% DM)	34.8^{a}	31.5^{b}	32.0^{b}	33.8^{a}
Gross energy (cal/g DM)	3973	4022	4022	3966

1) W · RS; Rice straw ensiled with water. LJ · RS; Rcie straw ensiled with intact LJ. LJ · NaOH · RS; Rice straw ensiled with NaOH treated LJ. LJ · NH_3 · RS; Rice straw ensiled with NH_3 treated LJ.

2) Means of 4 bag silages. Values in the same row with different superscript letters are significantly different ($p < 0.05$).

TABLE 3. CHEMICAL QUALITY OF THE SILAGES

Item	W · RS[1]	LJ · RS	LJ·NaOH·RS	LJ·NH_3·RS	Pooled SEM
Moisture (%)	74.1^{a}[2]	69.6^{b}	69.9^{b}	70.9^{b}	0.48
pH	4.67^{c}	4.77^{c}	6.02^{b}	7.03^{a}	0.05
Lactic acid (% DM)	0.09^{ab}	0.14^{a}	0.11^{a}	0.03^{b}	0.02
Acetic acid (% DM)	3.28^{c}	6.03^{a}	5.44^{b}	5.33^{b}	0.15
Propionic acid (% DM)	0.25^{c}	0.81^{a}	0.74^{ab}	0.63^{b}	0.05
iso-Butyric acid (% DM)	0.15^{c}	0.06^{d}	0.37^{b}	0.54^{a}	0.03
n-Butyric acid (% DM)	2.57^{c}	0.97^{d}	4.94^{b}	8.18^{a}	0.39
Total acids[3] (% DM)	6.72^{d}	8.18^{c}	12.1^{b}	15.7^{a}	0.41
Protein-N[4] (% DM)	0.53^{d}	0.66^{c}	0.74^{b}	0.81^{a}	0.02
NH_3-N/TN (%)	26.8^{c}	16.3^{d}	40.7^{b}	71.0^{a}	1.74

1) See the footnotes of table 2.

2) Means of 4 bag silages. Values in the same row with different superscript letters are significantly different ($p < 0.05$).

3) The sum of lactic and volatile fatty acids including valeric and capronic acids.

4) Trichloroacetic acid precipitable N determined with oven dried sample.

but it was still deficient to get a rapid lactic acid production which lead to a pH reduction. High buffering capacity of LJ might also prevent pH reduction because some buffering components were extracted into the juice by mechanical disintegration of leguminous plant (Ohshima and Oouchi, 1979). Acetate type fermentation might be attributable to a dominance of coliform bacterial growth caused under aerobic condition which lasted for a long period due to the use of tough straw as the silage material. The addition of NaOH or NH_3 treated LJ significantly increased the pH value of the silage with increase of butyric acid content and NH_3-N/TN. The LJ · NH_3 · RS silage, in particular, contained the lowest lactic acid and showed a butyrate type fermentation. The above results could be attributable to initial high pH value of the treated juice (table 1), additional buffering component with alkali and subsequent clostridial growth of the silage. The NaOH or NH_3 treated RS silage showed higher total acid contents than the other two RS silages. The increase of acid contents might be resulted from the increase of free carboxyl group contents by alkali treatment (Terashima et al., 1984). Protein-N content of RS silages was increased by the addition of LJ. Although the addition of NaOH or NH_3 treated LJ increased butyric acid and NH_3 N contents of the silage, protein-N content was not decreased. These observations were not consistent with general consideration in which clostridial fermentation causes protein and amino acid breakdown of silage (Ohshima and McDonald, 1978). The above results were in agreement with the experiment of Heinrichs and Conrad (1984) which showed that NH_3 treatment decreased proteolysis during ensilage while VFAs production shifted to less acetic and more butyric acid.

Results of digestion and nitrogen balance trial are shown in table 4. While crude protein digestibility of W · RS silage was higher than that of LJ · RS silage, the goats receiving the latter silage tended to excrete less urinary nitrogen and showed similar nitrogen retention to goats fed the former silage. Digestibilities of fibrous components of these two silages were almost the same and the values were quite similar to those of respective silages obtained in the previous study. The goats were provided more nitrogen with LJ

TABLE 4. DIGESTIBILITIES OF SOME NUTRIENTS AND NITROGEN BALANCE IN GOATS GIVEN THE 3:1 MIXED RATION OF RICE STRAW SILAGE AND WHEAT BRAN

Item	Diets containing				Pooled
	W · RS[1,2)]	LJ · RS	LJ · NaOH · RS	LJ · NH_3 · RS	SEM
Digestibility (%)					
Dry matter	$50.9^{b,3)}$	51.6^{b}	58.7^{a}	60.0^{a}	0.93
Organic matter	55.5^{b}	55.3^{b}	62.9^{a}	65.0^{a}	0.94
Crude protein	70.2^{b}	63.2^{c}	61.0^{c}	77.5^{a}	0.83
NDF	46.7^{b}	49.0^{b}	59.9^{a}	64.6^{a}	1.65
ADF	47.1^{c}	45.8^{c}	56.8^{b}	61.5^{a}	1.52
Hemicellulose	46.2^{b}	53.4^{b}	64.9^{a}	69.8^{a}	2.90
Cellulose	54.6^{c}	53.8^{c}	66.7^{b}	71.5^{a}	1.38
DE/GE[4)]	53.5^{b}	54.5^{b}	61.6^{a}	62.2^{a}	0.99
Nitrogen balance (g/day)					
Intake N	5.57^{a}	5.34^{b}	5.40^{b}	10.2^{a}	0.14
Fecal N	1.66^{d}	1.97^{c}	2.10^{b}	2.29^{a}	0.04
Urinary N	3.68^{b}	3.16^{bc}	2.74^{c}	6.61^{a}	0.20
Retained N	0.24^{b}	0.21^{b}	0.56^{ab}	1.30^{a}	0.28

1) See the footnotes of table 2.

2) Urea was supplemented at feeding time to adjust the nitrogen intake except for that of NH_3 treated silage.

3) Means of 4 goats. Values in the same row with different superscript letters are significantly different ($p < 0.05$).

4) Digestible energy/Gross energy.

than with GJ, however, the digestibilities of fibrous components of RS ensiled with water or intact juice were not different. There have been many evidences that the fiber digestibilities of low quality roughage were improved by supplementation with nitrogen. On the other hand, the improvement could be observed under relatively low level of nitrogen intake (8.5% of diet DM, Smith et al., 1980). Higher level of nitrogen supplement did not result a higher fiber digestibility (Liu et al., 1988). The extent of fiber digestion of the roughage was potentially limited due to the highly crystallized cell wall constituents such as lignin and silica (Kawamura et al., 1973). The above facts might suggest that energy availability of the roughage was considerably low for supporting microbial nitrogen incorporation into protein, and potential deficiency of nitrogen would be easily compensated with relatively low nitrogen intake. The cell wall constituents were swollen by the alkali treatment (Shinoda et al., 1984; Terashima et al., 1984), which could assure the microbial invasion into the inner tissues. According to the facts, the LJ · NaOH · RS and LJ · NH_3 · RS silages showed significantly higher digestibilities of fibrous components than the other silages. All the fiber digestibilities of LJ · NaOH · RS silage were lower than those of LJ · NH_3 · RS silage. However, the differences of NDF and hemicellulose digestibilities between NaOH treated and NH_3 treated silages were not significant, while in the previous experiment the differences were significant. The NH_3 treatment had a similar effect on improving the fiber digestibilities in the two experiments. These results suggested the potential fiber digestibilities of NaOH treated roughage were not fully shown in the previous experiment due to a nitrogen deficiency for rumen microbes but the deficiency was almost made up with additional nitrogen supply from LJ in this experiment. Takahashi (1985) also reported that the fiber digestibilities of NaOH treated roughage were improved with graded level of supplementary nitrogen.

Rumen fluid characteristics and BUN concentrations are presented in table 5. There were no significant differences of ruminal pH value, total VFA concentration and the VFA proportion among the goats fed four RS silages. However, compared with the goats receiving W · RS silage, those on the other RS silages tended to show higher ruminal total VFA concentrations suggesting increased energy supply from LJ added. Feeding LJ · NaOH · RS or LJ · NH_3 · RS silage appeared to further increase the concentration than LJ · RS silage feeding showing digestible components in the rumen were increased by the alkali treatment. Ruminal NH_3-N and BUN concentrations in goats fed W · RS silage were higher than goats on LJ · RS and LJ · NaOH · RS silages reflecting that urea was rapidly degraded in the rumen. The goats receiving LJ · NaOH · RS silage showed significant reduction of these concentrations compared with goats on W · RS and LJ · RS silages. These results might indicate that higher energy availability of LJ · NaOH · RS silage than those of W · RS and LJ · RS silages enhanced nitrogen incorporation into microbial protein (Sriskandarajah and Kellaway, 1984). The goats fed LJ · NH_3 · RS silage showed the highest concentrations of ruminal NH_3-N and BUN due to the highest nitrogen intake consisted mainly of ammonia. The ruminal NH_3-N concentration (13.8 mg/dl) of goats fed LJ · NaOH · RS silage was relatively high compared with the recommended level at which ruminal microbial protein production could be optimized *in vitro* (5 mg/dl, Satter and Slyter, 1974) or animals efficiently digested dry matter *in situ* (10 mg/dl, Leng, 1989). However, lower ADF and cellulose digestibilities of LJ · NaOH · RS than those of LJ · NH_3 · RS silage were obtained. This difference might be attributable to a nitrogen deficiency for rumen microbes from

TABLE 5. RUMEN FLUID CHARACTERISTICS AND BLOOD UREA NITROGEN (BUN) CONCENTRATIONS OF GOATS GIVEN THE 3:1 MIXED RATION OF RICE STRAW SILAGE AND WHEAT BRAN[1]

Item	Diets containing				Pooled SEM
	W · RS[2,3]	LJ · RS	LJ · NaOH · RS	LJ · NH_3 · RS	
pH	6.35[4]	6.37	6.44	6.46	0.06
Total VFA (mmol/dl)	6.63	7.68	8.68	8.81	0.85
Molar % of VFA					
Acetic acid	70.5	71.1	72.4	70.5	2.85
Propionic acid	20.4	18.7	17.3	16.5	1.69
iso-Butyric acid	1.10	1.05	1.00	1.00	0.14
n-Butyric acid	6.93	7.10	8.28	10.4	1.73
NH_3-N (mg/dl)	28.7^b	23.2^{bc}	13.8^c	57.1^a	3.10
BUN (mg/dl)	25.0^b	16.3^c	12.4^c	34.3^a	1.87

[1] Samples were taken 4 hours after morning meal.

[2] See the footnotes of table 2.

[3] Urea was supplemented at feeding time to adjust the nitrogen intake except for that of NH_3 treated silage.

[4] Means of 4 goats. Values in the same row with different superscript letters are significantly different ($p < 0.05$).

LJ · NaOH · RS silage. The NDF and hemicellulose digestibilities of NaOH treated RS silage were evidently improved in this study when compared with those in the previous experiment. These observations were probably because more nitrogen was supplied for the rumen microbes from LJ than from GJ. Therefore, ADF and cellulose digestibilities of NaOH treated RS silage could be further increased when animals were provided more nitrogen than that of present experiment. In conclusion, the addition of LJ at ensiling improved the chemical quality of RS silage, but did not show any advantageous effects on digestibility and nitrogen balance of goats compared with W · RS silage supplemented with urea at feeding time. When RS was ensiled with alkalized LJ, the fiber digestibilities were greatly increased. And the goats fed it was also improved their nitrogen balance through being provided more available energy than goats on untreated one for supporting nitrogen uptake into microbial cells in the rumen.

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