Utilization of Absorbed Nitrogen in Oxidized-Ammoniated Rice Straw and Rice Hulls by Rumen Microbes

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Chemical treatment with ammonia or both sodium chlorite and ammonia for rice straw and rice hulls markedly increases nitrogen content¹⁾ and improves the palatability and digestibility of these roughages by sheep²⁾. It has been shown in our previous reports^{3,4)} that the hot water insoluble nitrogen as well as non-protein nitrogen was increased by ammonia treatment and that the nitrogen content in fibrous materials was also increased. These results indicate that the increased nitrogen by the treatment exists as various nitrogen compounds and are retained at different place in roughages. The adsorbed nitrogen in low quality roughages originated from added ammonia may be utilized by rumen microbes for the synthesis of protein and subsequently stimulate rumen microbial growth. However, there is no information in this regard.

The present experiment was conducted to determine the effects of oxidized-ammoniated rice straw and rice hulls on ruminal activity as assessed by rates of the microbial protein synthesis and the adsorbed nitrogen incorporation, using rice straw and rice hulls treated with ¹⁵N labeled ammonia.

Materials and Methods

Rice straw (RS) and rice hulls (RH) were mixed with 50% water of air dry matter and than simultaneously treated with 0, 5 or 25% sodium chlorite (NaClO₂, by wt.) and 5% ammonia (NH₃, by wt.) containing 10.4 atom % ¹⁵N, at 45°C for 3 days. After removing excess ammonia by aerating for a few days, they were ground to pass the 1 mm screen in a Wiley mill. The treatments of NaClO₂ and NH₃ for RS and RH are detailed in another paper⁵⁾.

In vitro fermentation experiments were conducted using a 50 ml glass tube equipped with Bunsen valve top, to measure the microbial protein synthesis and incorporation of the adsorbed nitrogen into rumen microbes. About 0.5 g of sample was added

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as the substrate to each fermentation vessel containing 20 ml JOHNSON'S artificial saliva⁶⁾ and 10 ml rumen inoculum. Rumen fluid was obtained immediately prior to feeding from a fistulated sheep fed orchardgrass hay once daily and was strained through four layers of cheesecloth for inoculum. Fermentation trials were carried out in a water bath at 39°C for 24 hours. At the end of fermentation, the tube was placed in an ice water to stop the fermentation. The culture medium was then strained through four layers of cheesecloth, and the filtrate was analyzed for microbial protein nitrogen. Microbial protein was determined by sodium tungstate precipitation reported by SCHULTZ and SCHULTZ⁷⁾. The ¹⁵N concentration of the nitrogen was determined by the use of ¹⁵N analyzer (NIA-1, Japan Spectroscopic Co. Ltd). The data were subjected to analysis of variance and means were compared using Duncan's multiple range test⁸⁾.

Results and Discussion

The incubation medium contained 88.6 and 19.8 mg per 100 ml for total nitrogen and microbial protein nitrogen, respectively. Therefore, the 68.8 mg nitrogen except microbial nitrogen was to be available for the microbial protein synthesis. The amounts of nitrogen of microbial protein synthesized during the 24 hour incubation period was estimated by subtracting the initial value from the final value of incubation.

The microbial nitrogen contents of the treated sample were significantly higher than those of both original materials as shown in *Table*, suggesting that the ammoniated materials stimulated the rumen microbial activity. The values in RH were significantly increased as a function of the level of NaClO₂, but the value in RS treated with 25% NaClO₂ was significantly lower than that in the 0 and 5% NaClO₂ treated

Table 1. Microbial N synthesized during the 24 hour incubation period from the adsorbed N of rice straw and rice hulls treated with ammonia or sodium chlorite and ammonia

Treatment (% w/w) ¹⁾	Rice straw				Rice hulls			
NH ₃	0	5	5	5	0	5	5	5
NaClO ₂	0	0	5	25	0	0	5	25
Total N added as substrate (mg/100 ml)	8.5	28.5	31.1	33.8	7.5	24.2	27.6	27.6
Microbial N (mg/100 ml)	27.7 ^c ±2.1	42.5 ^a ±4.8	45.8 ^a ±1.3	34.5 ^b ±0.8	24.2 ^c ±1.2	27.2 ^{bc} ±0.8	28.7 ^b ±0.9	34.0 ^a ±3.8
The synthesized microbial N during incubation period (mg/100 ml) ³⁾	7.9	22.7	26.0	14.7	4.4	7.4	8.9	14.2
Microbial N derived from the adsorbed N (mg/100 ml) ³⁾ (% of synthesized N)		5.5 ^b ±0.6 (24)	7.0 ^a ±0.2 (27)	5.0 ^b ±0.1 (34)		1.5 ^c ±0.1 (20)	2.2 ^b ±0.1 (25)	4.8 ^a ±0.6 (34)

1) Air dry matter basis. 2) These values were estimated by subtracting the blank value (19.8 mg/100 ml) from that of each substrate. 3) Microbial N derived from the adsorbed N (mg) = Total microbial N (mg) × $\frac{^{15}\text{N atom \% excess in microbial N}}{^{15}\text{N atom \% excess in added NH}_3}$. Means in the same line with different superscripts differ significantly. (P < 0.05, means of 3 estimates ± SD)

ones. Further work is required to explain this discrepancy, although the higher level of NaClO_2 (25%) to treatment would be appear to give a detrimental effect on microbial activity. The stimulated effect of oxidized-ammoniated roughages on the synthesis of microbial protein would be due to the increases of available energy and nitrogen sources.

The microbial nitrogen derived from the adsorbed nitrogen was calculated by total microbial nitrogen content and ^{15}N concentration of microbial and added ammonia nitrogen as shown in *Table*. The value in the ammoniated RS treated with 0, 5 and 25% of NaClO_2 was 5.5, 7.0 and 5.0 mg/100 ml and 1.5, 2.2 and 4.8 mg in RH, respectively. These values in RH were significantly increased as the level of NaClO_2 increased. The values observed in RS treated with 0 and 25% NaClO_2 were similar and they were significantly lower than that of 5% NaClO_2 treated RS.

The rates of the microbial protein nitrogen derived from the adsorbed nitrogen to the total nitrogen of microbial protein synthesized during the incubation ranged from 24% to 34% for RS and from 20% to 34% for RH. These results indicated that the adsorbed nitrogen originated from added ammonia in roughages was certainly utilized by rumen microbes as well as other nitrogen sources.

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