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The disappearance of vitamin A from commercial sources during *in vitro* ruminal fermentation*

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ABSTRACT

The effects of three levels of vitamin A (5.8, 11.6 and 46.4 IU /ml of *in vitro* media) and two sources of vitamin A (Roche and JDW) on *in vitro* ruminal disappearance of retinol were studied. *In vitro* substrates that were similar to those fed to the donor dairy cows (containing 50% concentrate) were incubated with buffer ruminal fluid for 24 h. Retinol disappearance was affected by concentrations of vitamin A, and it was increased with the time of incubation, and was about 60% at 8 h and about 70% at 24 h. The degradation of retinol was not evidently affected by vitamin A sources.

KEY WORDS: vitamin A, disappearance, rumen fermentation, *in vitro*

INTRODUCTION

The importance of vitamins A on immune function is becoming well established, but only limited data reported the degradation of vitamin A by ruminants. Fernandez et al. (1976) suggested that only a portion of vitamin A has been quantified as reaching the small intestine in ruminant animals. In the early days, the researchers explained that the disappearance of vitamin A was caused by the chemical factors in rumen, whereas Rode et al. (1990) showed that when vitamin A passed through the rumen, a portion of vitamin A was degraded by microbes in rumen. The reason why it is more sensitive to degradation than the other vitamins is the conjugated double bonds of vitamin A, which are excellent electron sinks in the rumen environment. They also found that the bacterial strains capable of taking part in vitamin A metabolism are known to be associated with concentrates of diets. Weiss et al. (1995) showed that

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disappearance of vitamin A during *in vitro* ruminal fermentation was approximately 72% when donor cows were fed 50% concentrate diet.

However, Warner et al. (1970) reported that the effective vitamin A degradability was 65% for the high concentrate diet, and it was 55% for the high forage diet. Fewer data are available on the effects of supplemental dose and resources of vitamin A on its degradation in rumen. The objectives of the present study were to investigate the effects of different commercial sources and different levels of vitamin A on the ruminal disappearance of retinol during *in vitro* ruminal fermentation.

MATERIAL AND METHODS

Ruminal content collection, in vitro incubation, samplings and analysis

Two ruminally fistulated and healthy Holstein dairy cows (BW 550±25 kg) were used as donors of ruminal fluid. Lactating cows were fed a diet contained 50% concentrate (Table 1). The diet was balanced to meet NRC (2001) recommendations.

Table 1. Ingredient and chemical composition of diets fed to donor cows and substrates used in fermentation vessels, % of DM

Diets for donor cows	
Ingredient, %	
maize grain	27.73
soyabean meal	8.66
wheat bran	6.09
sesame meal	4.12
minerals and vitamins	3.13
maize straw	50.28
Chemical composition	
crude protein, % DM	11.69
NDF, % DM	45.20
net energy, MJ/kg DM	5.81
In vitro substrate ingredient, %	
maize grain	29.00
soyabean meal	9.50
wheat bran	6.00
sesame meal	5.50
maize straw	50.00

Ruminal fluid was collected *via* the fistula before feeding in the morning, and squeezed through two layers of cheesecloth into a 2-l flask, which was filled with CO_2 and kept at 39°C. Filtered ruminal fluid (20 ml) was mixed with 40 ml of buffer and 0.5 g of

air-dried substrate was added in each *in vitro* flask. Flasks were purged with CO_2, sealed with vented stoppers, and incubated in an environmental shaker (100 rpm) at 39°C. The remaining ruminal fluid was analysed to test the concentration of vitamin A. The buffer solution was similar as described by Weiss et al. (1995). Substrates for the incubations were similar to the diets fed to donor cows (Table 1), and all of them were ground through a 1-mm screen mill prior to mixing the *in vitro* diets. Substrates were incubated for 4, 8, 12 and 24 h at 39°C, respectively. Each incubation was conducted in duplicate at the same time.

At the end of each incubation time, flasks were swirled vigorously, and then 15 ml of contents were removed using a wide-tip pipette. Samples were mixed immediately with 10.5 ml of a solution of 0.5% pyrogallic acid in ethanol, and then were placed into a freezer (-20°C) until analysis. The following procedure was the same as described by Weiss et al. (1995).

Experimental design

The experiment was a 2×3 factorial arrangement of treatments. Three levels of vitamin A (350, 700 and 2800 IU) and two commercial sources of vitamin A (Roche Ltd. and Jin Da Wei Ltd. of China, JDW) were added to the flasks containing 60 ml *in vitro* ruminal fluid. At each of the incubation time, blank group was assigned, which in order to correct the concentration of vitamin A in *in vitro* ruminal fluid. The vitamin A was all-trans-retinyl acetate. The final concentrations of vitamins A in all *in vitro* media were 5.8, 11.6 and 46.4 IU /ml, respectively.

Statistical analysis

The data were analysed by analysis of variance with the levels and sources of vitamin A as main effects (SAS, 1998). A level of $P<0.05$ was used as the criterion for statistical significance.

RESULTS

Sources of vitamin A did not affect disappearance of retinol ($P>0.05$), but the disappearance of retinol from Roche was slightly lower than those from JDW, and it ranged from 45.62 to 74.85% for the different incubation time points (Table 2). *In vitro* disappearance of vitamin A increased with the time, and it was above 40% (45.6 and 41.3%, respectively) at 4 h, up to about 60% (59.8 and 71.9%, respectively) at 8 h, and above 70% (74.9 and 76.2%, respectively) at 24 h.

Table 2. Effects of vitamin A sources on disappearance of retinol *in vitro* incubation system, %

Vitamin A source	Incubation time, h			
	4	8	12	24
Roche	45.62 ± 12.86[a]	59.84 ± 11.03[a]	62.22 ± 4.38[a]	74.85 ± 11.02[a]
JDW	41.29 ± 20.88[a]	71.88 ± 15.28[a]	70.67 ± 12.24[a]	76.16 ± 13.85[a]
P value	0.50	0.23	0.10	0.68

[a,b,c] means within the same column without the same superscripts differ significantly (P<0.05)

Disappearance of vitamin A was affected significantly by *in vitro* ruminal vitamin A concentrations (Table 3). The adding of 5.8 IU/ml of vitamin A *in vitro* ruminal fluid resulted in the greatest disappearance of retinol at 24 h and the least disappearance of retinol at 4 h compared with the adding of 11.6 and 44.6 IU/ml of vitamin A. In addition, disappearance of vitamin A for different vitamin A concentrations of ruminal fluid also increased with the incubating time and was about 40% at 4 h and was up to above 60% at 8 h.

Table 3. Effects of vitamin A concetrations on disappearance of retinol *in vitro* incubation system, %

Vitamin A IU/ml	Incubation time, h			
	4	8	12	24
5.8	24.27 ± 8.82[a]	67.55 ± 11.47[a]	55.56 ± 4.09[a]	90.64 ± 4.30[a]
11.6	49.87 ± 10.32[b]	68.47 ± 20.54[a]	73.49 ± 10.52[b]	73.78 ± 5.34[b]
46.4	58.36 ± 5.23[b]	61.58 ± 7.72[a]	66.51 ± 1.83[ab]	62.09 ± 3.47[c]
P value	0.0054	0.810	0.060	0.0002

[a,b,c] means within the same column without the same superscripts differ significantly (P<0.05)

DISCUSSION

Weiss et al. (1995) examined that the disappearance of vitamin A of different commercial forms during 24 h *in vitro* incubation. They found that there was no effect of forms of vitamin A on disappearance of retinol, but not the same at different time points. After 24 h of incubation, 26% of added retinol was recovered for the 50% forage diet, and 80% of it was recovered for the 80% forage diet in that study. These results suggested that degradation of vitamin A might be affected by the type of diet.

Rode et al. (1990) showed that the bacterial strains capable of taking part in vitamin A metabolism were the most active bacteria to the digestion of starch, and bacteria associated with forage diets were incapable of taking part in vitamin A metabolism.

In the current study, the results indicated that the disappearance of vitamin A was about 60% for two sources and three levels of vitamin A after 8 h of incubation when *in vitro* diet contained 50% concentrate. Data from the present experiment

suggest that high concentrate diet will result in a greater disruption of vitamin A. It may be necessary to increase the provision of vitamin A of lactating dairy cows or to protect vitamin A from degradation of microorganisms in the rumen or to reevaluate the vitamin A requirement for lactating dairy cows. In addition, the present study also showed that the concentrations of vitamin A during *in vitro* ruminal fermentation affected the degradation of retinol , but the probable reasons were not clear.

CONCLUSIONS

It is concluded that concentrations of vitamin A affect significantly retinol disappearance, and sources of vitamin A do not affect disappearance of retinol. The degradation of retinol was increased with the time of incubation, and was about 60% at 8 h and about 70% at 24 h for *in vitro* diet containing 50% concentrate.

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