In Vitro Destruction of Vitamin A by Abomasal and Ruminal Contents

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Losses observed after incubating known quantities of vitamin A in ruminal fluids anaerobically at 37° C. for 4 hours averaged 36.1% as compared with 13 and 16% of the same quantities incubated in autoclaved ruminal fluid and distilled water, respectively. Incubation of vitamin A in abomasal fluids under similar conditions resulted in losses which averaged 33.6%. Thus, both the rumen and abomasum appear to have significant roles in the preintestinal destruction of vitamin A.

KLATTE *et al.* (*4*) observed extensive pre-intestinal disappearance of vitamin A in sheep. Since blood data appeared to rule out absorption, the disappearance was attributed to destruction; but the site(s) or mechanism(s) of destruction were not determined. The work reported herein was conducted to estimate the individual roles of the rumen and abomasum in vitamin A destruction.

Procedure

Known quantities of vitamin A were incubated in vitro with ruminal and abomasal fluids, and disappearance was measured. Steers and wethers with ruminal and abomasal fistulas were used as sources of fluids. All animals were maintained on vitamin A–free diets, soybean oil meal, and low carotenoid oat straw, to minimize carotene interference with vitamin A analyses and to avoid possible variability in the contribution of dietary components to the vitamin A content of the experimental fluids. Feeding of the experimental diet was initiated 1 week prior to the initial fluid withdrawal and continued throughout the 120-day experimental period. Collections of ruminal fluid were made via fistulas by taking approximately 1000 ml. of contents by a random grab sampling technique. Collections of abomasal fluid ranging from 400 to 800 ml. were obtained via abomasal fistulas.

Immediately after sampling, the samples from both the rumen and the abomasum were strained through double layers of cheesecloth, and the filtrates were centrifuged at low speed to remove heavier feed particles. The pH of each sample of fluid was determined. In each trial, aliquots of 10 ml. of the test fluid (ruminal fluid, abomasal fluid or distilled water) were immediately pipetted into each of six 40-ml. test tubes. One milliliter of aqueous 20% Tween "80" solution containing 20 I.U. of vitamin A acetate was added to each tube. Three of the tubes were analyzed for vitamin A immediately; and the remaining three were incubated under carbon dioxide at 37° C. for 4 hours, after which the samples were analyzed for vitamin A. The vitamin A analysis was made by an adaptation of the method of Johnson (*2*), using 0.75*N* alcoholic KOH for saponification, petroleum ether for extraction, and $SbCl_3$ in $CHCl_3$ for color development. Forty-one trials were conducted using distilled water, ruminal fluid from both steers and wethers, abomasal fluid from steers and wethers, and autoclaved ruminal fluid.

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Table I. Recovery of Vitamin A[a] after 4 Hours in Distilled Water, Ruminal Fluid, or Abomasal Fluid

Incubation Fluid	Number of Trials[b]	Average Recovery (%)
Distilled water	11	84.0 ± 3.1[c]
Steer ruminal fluid	7	64.4 ± 6.8
Wether ruminal fluid	4	63.0 ± 1.9
All ruminal fluids	11	63.9 ± 4.2
Autoclaved ruminal fluid	2	87.0 ± 2.0
Steer abomasal fluid	7	69.0 ± 7.2
Wether abomasal fluid	10	64.8 ± 4.7
All abomasal fluids	17	66.4 ± 3.4

[a] 20 I. U. of vitamin A acetate was added to each tube. [b] Each trial consisted of 3 control and 3 incubated tubes. [c] Standard error.

Results and Discussion

The distribution of the trials and the results of incubation in each fluid are summarized in Table I. Little difference was noted between recovery of vitamin A from the ruminal fluid of steers and ruminal fluid of sheep. The combined values of the 11 trials showed a recovery of 63.9%, or a destruction of 36.1%. This was 20.1% destruction in excess of that observed in distilled water. Illinois workers (*3*) reported average in vitro recovery of vitamin A incubated with ruminal fluid for 9 hours to be 64.4%. A preliminary report of work at the New York station (*1*) indicated that 75 to 80% of the vitamin A was recovered from ruminal fluid after 4 hours. The recovery from autoclaved ruminal fluid was similar to the recovery from distilled water; thus, a significant role of active microflora in the destruction of vitamin A in the rumen is suggested. However, autoclaving may have inactivated other biological factors with destructive activity. Variations in pH of the ruminal fluids (6.2 to 6.8) were not closely related to variations in vitamin A destruction. Changes in destruction also appeared randomly distributed over the 120-day experimental period, indicating that long-term adaptation to the experimental diet did not affect the destructive mechanism(s).

The average recoveries of vitamin A from abomasal fluids are surprisingly similar to those found in the trials with ruminal fluid. Limited work has been reported by other stations on the effects of abomasal fluids on vitamin A. Missouri workers (*6*) reported extensive losses of carotene in aqueous dispersions in the presence of nitrate at pH's of 1 to 3. This pH range approximates that of abomasal fluid (2.6 to 3.5 in the present study). South Dakota workers (*5*) reported complete destruction of vitamin A in abomasal fluids in the presence of nitrates.

These results indicate that both the rumen and abomasum have significant roles in the preintestinal destruction of vitamin A. Although the recoveries from ruminal fluid and abomasal fluid after 4 hours were similar in this work, this should not be interpreted to mean that the rumen and abomasum have equal roles in the destruction. Relative time the ingesta spend in the rumen and abomasum would be a major factor in this evaluation. It also seems likely that different mechanisms of destruction are involved. Further study of these mechanisms is needed.

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PLANT NUTRITION AND FEEDING VALUE

Some Nonfermentable Free Sugars in the Leaf-Petiole Fraction of Alfalfa *(Medicago sativa)*

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Sucrose, glucose, and fructose, which are relatively abundant and which interfere with the detection of less abundant sugars, were removed from prepared extracts of alfalfa by yeast fermentation. The nonfermentable free sugars isolated from the leaf-petiole fraction of alfalfa and identified by paper chromatography were D-*glycero*-D-*manno*-octulose, galactose, *manno*-heptulose, arabinose, *altro*-heptulose (sedoheptulose), xylose, and ribose. *manno*-Heptulose was also identified by its x-ray powder diffraction pattern. An additional unknown ketose-reactive material was separated but was not identified. Information concerning the natural occurrence of these sugars provides a basis for examining qualitative and quantitative changes which may occur as a result of altering the nutrition of the plant and which may affect palatability of the hay.

One of the many factors which determine the acceptability of forage by livestock is the sugar content. Plice (*21*) reported several comparisons in which the preference by cattle for different feeds was attributed to differences in their sugar levels. The differences in sugar levels were the result of variations in the conditions of growth of the forage plants or of additions of sweetening solutions of sucrose, molasses, etc., before the feeding. More recently, Bland and Dent (*3*) reported a significant positive correlation between the preference by cattle for several varieties of cocksfoot and their total sugar content. Similar results were obtained in other studies with sheep. Harper and Elvehjem (*9*) reviewed the literature on the influence of dietary carbohydrates on vitamin and amino acid requirements. They concluded that "indirect effects of individual carbohydrates may be of considerable nutritional significance."

The free sugars present in greatest amounts in most forages are sucrose, glucose, and fructose. Small amounts of other free sugars are also present, but prior to the advent of paper chromatography, they could not be readily separated and detected. Some of these, particularly in their phosphorylated form, have been assigned important roles in plant metabolism. Others, such as the oligosaccharides, melibiose, raffinose, and stachyose, have been found in grasses (*20*), but no specific metabolic function has been defined for them.

The purpose of the present study was to identify some of the nonfermentable free sugars which are present in the leaves and petioles of alfalfa. Yeast fermentation of the prepared plant extract removes sucrose, glucose, and fructose which interfere with the detection of some of the less abundant compounds. These studies are part of a project dealing with the effects of mineral nutrition of plants on their composition and feeding value. The authors have reported earlier (*22*) that alfalfa plants grown in solutions containing amounts of sulfur inadequate for maximum growth contain lower amounts of sucrose, glucose, and fructose than do similar plants grown in adequate nutrient solutions. The present findings will allow an extension of this study to include other carbohydrates. Information regarding the natural occurrence of these less common sugars also will provide clues regarding likely biosynthetic pathways to help elucidate, for example, earlier observations regarding the biosynthesis of heptuloses from pentoses introduced into plant tissue (*24*).

Experimental

Young shoots (regrowth about 10 days after cutting) of field alfalfa (Caliverde variety) were collected, and the leaves and petioles stripped from the stems and placed directly into boiling 95% ethyl alcohol. The procedure used for extraction and for subsequent yeast fermentation was that of Williams and Bevenue (*29*). Since, under some conditions, a ketoheptose can be synthesized during fermentation of hexose and ketose monosaccharides with yeast juice (*25*), the possibility that artifacts of yeast metabolism might be synthesized was checked. When yeast was allowed to act on a substrate consisting of 2% sucrose, 1% glucose, 0.66% fructose, and 0.01% xylose and ribose, no new sugars could be detected.

Whatman 3MM chromatographic paper sheets were used for isolation, and Whatman No. 1 paper for chromatographic detection. For one-dimensional chromatography, irrigant A [ethyl acetate–pyridine–water (8:2:1 v./v.)] was employed. For two-dimensional tests, irrigant B [phenol–water (10:2 v./v.)] was used for the second irrigation. Isolates from the alfalfa were cochromatographed with authentic samples of sugars by two-dimensional chromatography. Chromatograms were developed by dipping in orcinol (*2*), aniline (*16*), or silver nitrate (*26*) reagents. The resins used for clarification of the sugar solutions were Amberlite IR 120 (H^+) and Duolite A-4 (OH^-).

The fermented alfalfa extract was subjected to preliminary chromatographic examination, and sugars with R_G($R_{glucose}$)