INFLUENCE OF CARBOHYDRATE SOLUBILITY ON NON-PROTEIN NITROGEN UTILIZATION IN THE RUMINANT¹,²

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INTRODUCTION

Although soluble carbohydrates and carbohydrate solubility may sound synonymous, they, in fact, are not when one considers their influence upon the fermentation in the rumen. The former refers to a restricted class of carbohydrates of specified solubility whereas the latter portrays a characteristic of any carbohydrate or group of carbohydrates that often can be modified by pH and concentration as well as by chemical, physical, and microbial processing techniques. As such, the latter characteristic becomes more critical in a discussion of effects on non-protein nitrogen (NPN) utilization.

This review is in no way an exhaustive review of the effects of all forms of carbohydrates or NPN utilization. The subject of NPN utilization has been reviewed many times, most recently by Helmer and Bartley (1971). Far too many of the literature citations which could be cited represent repetitive findings which contribute very little definitive or new information to our information bank on NPN utilization. References quoted herein are primarily for the purpose of setting the stage for the ideas and challenges to be presented. In effect then, the purpose of this paper is to stimulate the future investigators of this topic to (1) investigate newer, more novel areas of research, (2) accept the challenge of improving research techniques so that really new information can be obtained using them, and concurrently, (3) abandon those techniques which, although traditional, are not particularly definitive and are often a waste of time and effort.

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CARBOHYDRATES TO BE CONSIDERED

Although numerous forms of carbohydrates exist in the feedstuffs ordinarily used in association with NPN, they can be classified into three major classes on the basis of their rates of digestion in the rumen. These are (1) cell wall carbohydrates such as cellulose and hemicellulose, (2) readily fermented forms of glucose polymers such as starch and dextrins and (3) various forms of simple sugars such as those that exist in molasses. Although this classification may seem presumptuous, it is necessary to simplify the discussion related to NPN utilization and to understand research results concerning the problem.

Figure 1 illustrates the reason for making this over-simplified classification. This presents a theoretical graph of the fermentative activity resulting from the ingestion of these three classes of carbohydrates into a rumen fermentation system. From a practical point of view, for example, any point on the curve might represent rate of gas production or organic acid production at that moment in time. It should

![Figure 1: Illustrations of the theoretical rumen fermentation rates overtime after ingestion of three forms of feed carbohydrate. A = soluble sugars, B = starches and dextrins and C = cell-wall carbohydrates.](image)
be noted that the fermentative curves for the three classes of carbohydrates differ in two striking characteristics, the first one being the amplitude of the fermentative curve and, secondly, the time span for that activity. This, of course, is no different than simply saying that cellulose is digested slowly over a long period of time, and sugars are digested rapidly over a short period of time, and starches are some place in between. It has classically been concluded by several reviewers and workers in this field that the fermentation of starch or starch feedstuffs favors the utilization of non-protein nitrogen over both the fermentation of fibrous carbohydrates and simple sugars such as molasses. From a theoretical basis this is difficult to explain for reasons which will be discussed.

**UTILIZATION OF AMMONIA**

Let us assume for the moment that the majority of rumen microorganisms utilize nitrogen which has passed through the ammonia pool in the rumen. Although this is not entirely true it has been estimated that, except for by pass protein, somewhere between 60 and 80% of the nitrogen passes through this NH₃ pool. If this is true for all forms of carbohydrate, then we can assume that the ideal situation in the rumen in regards to the ammonia pool would be that it demonstrates a curve somewhat similar to the carbohydrate fermentation curve, (Thomas, 1973; Blackburn, 1965) resulting in something like the picture in figure 2. In this figure, ammonia curves have been superimposed upon the fermentation curves illustrating the point just made. The ammonia release patterns from most non-protein nitrogen sources, and certainly crystalline urea, conform to the pattern for source “x”. Therefore, at the moment, no way can be visualized for NPN sources to conform to the needs for all three types of carbohydrates, assuming the animals are fed only once or twice daily. Inherently, the assumption is also being made that the groups of microorganisms which utilize the three classes of carbohydrates all have the same ability or requirement to utilize nitrogen which is passed through the ammonia pool as the major source of nitrogen. This may or may not be a valid assumption as most rumen microbiologists would agree that there is not sufficient data available as yet about the host of rumen microorganisms to make this a firm conclusion. According to Bryant (1973) virtually all of the important cellulose digestors can utilize ammonia as their major nitrogen source and in many cases, as the only nitrogen source. Likewise, most of the major starch digesting species as cited by Hungate (1966) can utilize ammonia as their major nitrogen source. Many of these species may have other requirements for specific amino acids or peptides as other forms of nitrogen, but virtually all of them are capable of utilizing ammonia to a high degree. The microorganisms responsible for the short term digestion of more soluble carbohydrates such as found in molasses have not been as thoroughly investigated or at least discussed in previous reviews as far as NH₃ utilization and are an area worthy of additional research. If *Streptococcus bovis* is a major simple sugar utilizer, which may well be the case, this species is a well-known ammonia utilizer. There is little question that all classes of carbohydrates can be digested using NH₃ as the major N-source since the work of Oltjen (1969) and Virtanen (1966) showing how long ruminant animals could survive on purified rations containing only urea as a N-source, although symbiosis or the ability of one microorganism to live on the nitrogenous compounds produced by another organism may explain part of that observation.

There is little question but what molasses fermenting microorganisms in animals adapted to molasses can utilize urea as a source of nitrogen. This has been well proven, by the
Cuban workers (Munoz et al., 1970; Ramirez, 1972; Ramirez and Kowalczyk, 1971) in the use of rations consisting of more than 50% molasses in which urea has been utilized as the major source of supplementary nitrogen. To my knowledge, however, the rumen microbiology associated with this high molasses ration has not been investigated so this may not represent a situation typical of the somewhat more normal circumstances where small percentages of molasses are included in rations. This certainly bears further investigation.

On the surface, therefore, no logical explanation can be seen why the utilization of starch should favor urea or NPN utilization to a greater extent than the inclusion of simple sugars or molasses. It must be borne in mind, however, that in many of the comparisons made the quantities of fermentable carbohydrate added in the form of starch and in the form of molasses were not necessarily the same.

**CARBOHYDRATE COMPETITION**

In the presence of different forms of carbohydrate there is obviously a competition for the available nitrogen sources between the groups of microorganisms utilizing the different forms of carbohydrates. This fact was well illustrated by the data accumulated by El Shazly et al. (1961) in which they showed that in the presence of both starch and cellulose in purified forms, cellulose digestion was depressed when urea was utilized as a source of nitrogen. On the other hand, in the absence of starch considerably more cellulose was digested. Although this could represent competition for a number of nutrients besides nitrogen, some of this competition could be alleviated by simply adding additional nitrogen as urea to the system. This was demonstrated by both in vitro and in vivo techniques. Similar studies with molasses were not conducted.

**MOLASSES-UREA COMBINATIONS**

Numerous experiments have been conducted in studying the effect of combinations of molasses and urea as stimulants to the utilization of low quality roughages in Australia, South Africa, the U.S.A., and other areas. In general these supplements improved the consumption rate of the available low quality roughage without affecting its digestibility, thus, suggesting an increased rate of digestion in the rumen. Interestingly enough, however, this effect was brought about as often as not with urea alone as when the molasses and urea were supplied in combination. Because of the rapid hydrolysis of urea to ammonia and the slow digestion of low quality roughages this result could only have come about by the well known recycling system for nitrogen in the ruminant. Unfortunately, the efficiency of the nitrogen recycling system in ruminants has not been quantified under a variety of nitrogen status circumstances. The fact that molasses many times did not improve animal performance under such circumstances suggested that there was very little microbial protein synthesis due to the molasses fermentation, which supports the earlier statement that molasses may not be the best source of carbohydrate for stimulation of NPN utilization.

Possibly no other development has received more marketing and sales success in this country than the liquid supplement industry. Regardless of the many materials used to formulate liquid supplements, they can be considered, in general, combinations of molasses and urea. From the standpoint that their usage is designed for low quality roughage rations, they should be looked upon primarily as sources of nitrogen and energy. The story of their use in the field is fraught with testimonials of both success and failure, often without any apparent reason for one or the other. One of the most obvious reasons for lack of solid proof of efficacy in field studies is the fact that, in many cases, supplements are being used with rations that require no nitrogen supplementation to start with. What is more important is that, unfortunately, like many of the research trials, the field trials involved no comparison with a negative control to determine how well the animal might have performed without a nitrogen supplement. This represents one of the travesties of justice within our scientific discipline which is inexcusable. The liquid supplement industry flourishes whether it be right or wrong without the benefit of sufficient soundly designed, definitive scientific research on the abilities of these materials to foster rumen microbial protein synthesis. Although this could also have been said about much of the older urea research, the scientific community should have known better by the time liquid supplements began to be tested.

**SUSTAINED RELEASE FORMS OF NITROGEN**

From a theoretical standpoint, again observing the theoretical curves in figure 2, an ideal
source of nitrogen to the ruminant consuming rations containing all three carbohydrates would be one which would support release of nitrogen through the ammonia pool on a rather steady basis throughout all of those periods of time involved, or at least in magnitudes coinciding with the fermentative activity. However, this is actually a false assumption in terms of the way carbohydrates are distributed in many of the feedstuffs utilized in conjunction with NPN supplements.

Figure 3 gives a more realistic representation of the levels of the three classes of carbohydrates present in a regime of low quality grass, grain, and molasses supplement. In this figure, it is obvious that the rumen NH₃ curves do not match the fermentation curves in magnitude. The NH₃ curves resulting from many typical supplement combinations might well look something like those shown here so it is not unexpected that NPN utilization might be somewhat less than maximum under these circumstances.

Actually, the fermentation curve should probably be drawn as shown in figure 4 to be more realistic. Then N-supplements might be designed to fit them. This may well be achieved by various combinations of nitrogen including combinations of urea, protein meal supplements, and cereal grains. Indeed, proteins of widely varying solubilities in natural feedstuffs may satisfy this requirement. However, it is the desire from the economic standpoint, as well as the theoretical purpose for this review, to design methods for increasing NPN utilization. Since at the moment there are no NPN sources which to the author's satisfaction meet the criteria illustrated in this theoretical situation then the necessity for developing a sustained release form of urea becomes obvious. In fact, it would seem likely that the successful use of "bypass protein" together with maximization of NPN utilization to achieve as close as possible the upper limits of microbial protein synthesis would necessitate this type of development of a sustained release product.

Numerous products have been tested, both in the author's laboratory (Johnson et al., 1962; Johnson and McClure, 1968) and others, which represent various forms of coatings applied to urea particles in efforts to sustain the release of these materials. From this work it was obvious that the major process of sustaining the release was that of simply shielding the urea from solution in the aqueous media in the rumen. Once the material was dissolved, of course, it was almost immediately hydrolyzed. Although numerous products appeared to have possibilities, at the time that they were investigated economic conditions did not suggest that further development to a marketable product was merited.

Because of more recent emphasis on the use of NPN sources, however, we decided to test the theoretical soundness of this hypothesis in
our laboratory by simulating a sustained release system. In these studies an automatic feeder designed to feed the animal every hour throughout the 24-hr day was utilized to provide this stimulation. These data have been reported earlier (Males et al., 1974). Microbial protein synthesis was estimated when urea contributed 0, 25, 50, or 75% of the supplementary-N to a low quality roughage ration with a negative “urea fermentation potential”. Under these circumstances of relatively steady input of NH₃, the utilization of NPN from urea for microbial protein synthesis was almost as efficient as the utilization of nitrogen from soybean meal. In fact, it appeared that the major limitation of microbial protein synthesis was that of energy in the ration rather than the nitrogen source. This indeed sustained the earlier theory and stimulated the design of several other experiments presently underway, concerning both level of energy and form of energy. The fact was, however, that NPN was being utilized for microbial protein synthesis on a very low quality roughage ration.

**Proposed Sustained Release Products.** Several products have appeared on the market in recent years which are reported to have been formulated to increase the utilization of urea as a source of nitrogen. These vary from the usual liquid supplement to various combinations of urea and processed starch. It should be emphasized that the processing of starch primarily increases the rate of fermentation of this material in a way illustrated in figure 5. It can be seen in this figure that the processing technique increased the rate of fermentability and simply shifts the fermentative curve for starch to the left and changes the shape slightly. It probably does not drastically change the group of microorganisms which are digesting the starch, however. Nevertheless, there are important changes taking place in both pH and rumen ammonia when these forms of carbohydrate are ingested by the animals.

Figure 6 illustrates the pH curve in sheep fed a straight corn-urea mixture compared to an isocaloric and isonitrogenous supplement made up of either a typical liquid molasses-urea supplement or processed forms of starch and urea (J. R. Males and R. R. Johnson, unpublished data). The important conclusion from these graphs is that the pH peak following this amount of urea ingestion is considerably lower in the presence of either molasses or the processed forms of starch as compared to the native starch in the ground corn. Figure 7 illustrates the rumen ammonia curves resulting from these same studies. It can be seen that there was actually no marked difference in the amount of ammonia present in the rumen immediately following the solution and hydrolysis of the urea. However, in figure 8 it can be observed that the blood ammonia curve was drastically different for the different forms of carbohydrate and urea.

These data support the conclusion that the absorption of ammonia from the rumen is much more dependent upon the pH of the rumen than upon the actual total amount of ammonia in the rumen. Of course, this may
have more relation to the toxicity of various products than it has to the actual efficiency of utilization of the nitrogen, but it would seem that there is some bearing on the latter item as well. Limited nitrogen balance data in this laboratory and considerably more nitrogen balance data in the Kansas State University laboratories (Shiehzadeb and Harbers, 1974) suggest that the processed starch-urea forms do promote greater utilization of the nitrogen than the simple mixtures of corn and urea. The solid proof of this has been accomplished primarily with high energy rations, however, since they will support greater nitrogen balance. The relative efficiencies of the utilization of their nitrogen contents when fed with low quality roughages rations has yet to be determined and will probably rely on the measurement of the synthesis of microbial protein rather than on nitrogen balance or other classical measurements.

Figure 7. Rumen NH₃ in lambs following consumption of single doses of various carbohydrate-urea combinations.

In the interpretations of results, the role of protozoa in the utilization of non-protein nitrogen in the rumen bears some consideration. This is a role which has been researched to only a limited extent, and it is absolutely essential that this role be investigated further before a clear understanding can be obtained. For example, to date there is no clear explanation why the rumen ammonia levels in defaunated animals are so much lower than they are in faunated animals (Males and Purser, 1970). Furthermore, the relationship of the soluble carbohydrate forms to the protozoa population may be subtle but extremely important. It is well known (Hungate, 1966), for example, that the Holotrichs are basically sugar utilizers and, therefore, their population must assuredly be affected by the presence of soluble sugars. On the other hand, the Entodiniomorphs are predominantly starch digesters, and, of course, their magnitude might be directly related to the form of starch being ingested. In a recent review by Miller (1973), it was stated that the protozoa can vary from 5 to 44% of the total protein in the rumen. This might easily be modified to 0 to 44% since many animals on high concentrate rations exist as defaunated animals. In any biological ecosystem where one component can contribute as much as 44% of the protein, obviously this component has to be considered more seriously than the protozoa have been in their relationship to utilization of any form of nitrogen.

Figure 8. Plasma urea nitrogen (PUN) in lambs following consumption of single doses of various carbohydrate-urea combinations.

PROCESSING EFFECTS

The site of digestion of the carbohydrates in the ration has to be considered also. Obviously the cell wall carbohydrates will have to be digested primarily in the rumen and it is probably a safe conclusion that the soluble carbohydrates are almost 100% fermented prior to leaving the rumen. The site of digestion is of primary concern only in relation to the starch entering the rumen. It is well known that the degree of processing by modern techniques for grain will influence the amount of starch which is digested in various portions of the tract. In addition, however, recently Ørskov et al. (1971) showed that barley starch was almost 100% digested in the rumen but, in contrast, corn starch was digested to a much greater extent in the lower tract. This simply throws additional caution on the interpretation of
research on the effect of carbohydrate source on the efficiency of utilization of NPN.

DISCUSSION AND CONCLUSIONS

Future research on the utilization of non-protein nitrogen forms, regardless of what they are, will have to be far better designed than many of the studies reported in the literature today. The research techniques required for this purpose have enjoyed considerable development over the last 10 years and, in general, most of them are appropriate to most animal science laboratories of this day and age. For example, the use of animals cannulated in various portions of the digestive tract other than the rumen will facilitate measurement of the processes which have occurred in the rumen itself in order to isolate those from the occurrences in the lower digestive tract. A number of materials are available as internal or external markers which will facilitate the calculation of total protein passage. In addition, there are now several techniques which have been proposed for the measurement of microbial protein as a fraction of the total protein passing through the tract. The use of RNA, diaminopimelic acid and ciliatine are all applicable to these studies and should be investigated. No laboratory study involving a calculation of the efficiency of utilization of a non-protein nitrogen source should be conducted without the use of these techniques in one form or another.

Nitrogen balance studies are in general not very discriminatory in the determination of efficiencies of various nitrogen sources for ruminant animals. The use of the depletion-repletion techniques suggested by Biddle and Evans (1972) has improved the discriminatory ability of nitrogen balance techniques but still would leave something to be desired. In those studies involving low quality roughage rations, nitrogen balance techniques have proven to be fruitless since the balance is near zero or below on most of the regimen being utilized. The coefficients of variation around means at this level are virtually unmanageable. This then suggests that in these circumstances the more sophisticated techniques of measuring microbial protein synthesis are almost mandatory.

This is not to suggest that field research or applied research, if that term is chosen, is not appropriate. However, it is safe to say that the data resulting from much of the applied research studies in the past on non-protein nitrogen are subject to a variable interpretation. Certainly if they are to be conducted in the future, they should be designed with sufficient numbers of controls to allow interpretation of the data to the extent of concluding whether the NPN source was actually utilized as a source of nitrogen or not. The equal performance of animals on an NPN source relative to performance on another source of nitrogen is not sufficient evidence to conclude that one was utilized as well as the other. This is essentially what exists in many papers in the literature.

During the severe economic cycles in ruminant livestock production, there have been several periods when the use of NPN was increased dramatically because of favorable cost margins compared to natural protein sources. Most experienced NPN researchers would have been hesitant to advise this in the case of supplements to low energy rations, based on the lack of solid proof of their efficacy in those circumstances. Surely, there could be no greater contribution to ruminant nutrition than for scientists to define in the near future the conditions under which NPN utilization can be maximized. These conditions will no doubt relate in a large measure to the proportions of carbohydrates of varied solubility.

LITERATURE CITED


Males, J. R. and D. B. Purser. 1970. Relationship between rumen ammonia levels and the microbial


