

How Do pH and Protein Source Affect Nutrient Yield From Sucrose Fermentations?

Mary Beth Hall, Associate Professor, Department of Animal Sciences, University of Florida
Lucia Holtshausen, Graduate Student, Department of Animal Sciences, University of Florida

Objective: The purpose of these experiments was to evaluate how pH or protein source in a fermentation affected the amounts of nutrients (microbial protein, organic acids, glycogen) produced from sucrose by rumen microbes.

Introduction

We performed fermentations of sucrose with rumen microbes in the lab to examine how pH or protein source affected the types and yields of fermentation products useful to the animal. The fermentations with starting pH of 6.7 or 5.7 would be representative of animals with relatively neutral pH (high forage ration) vs. those that are more acidic (high grain ration). In another set of fermentations, protein was provided by all non-protein nitrogen, all true protein, or a blend, all at a starting pH of 6.7, to represent different protein supplementation schemes. The in vitro fermentations used sucrose + bermudagrass neutral detergent fiber and bermudagrass NDF alone (120 mg of sucrose with 120 mg of bermudagrass NDF, or 240 mg of bermudagrass NDF in 50 ml tubes with gas release valves). Bermudagrass fiber was included to sustain a greater variety of rumen microbes than would be supported by sucrose alone. Two separate fermentation runs were performed per study. Goering and Van Soest medium was used for the protein study, and for the 6.7 pH of the pH study. Goering and Van Soest medium with citric acid added to decrease the pH to 5.7 was used for the low pH treatment on the pH study.

A variety of products/measures were examined over the courses of 24 hour (pH) or 16 hour (protein) fermentations to get a better idea of what changes in nutrients available to the animal may occur over time.

Measures:

Microbial protein represents an excellent source of protein for the animal. It was measured as trichloroacetic acid (TCA) precipitated crude protein (TCACP) in a sample corrected for the fermentation blanks for that sampling hour and for the TCA precipitated protein of the sample itself calculated from 0 hour data. This is a conservative estimate of microbial protein yield because it assumes that no TCA-precipitable protein in the sample was used by the microbes.

Sucrose, glucose, and fructose measurements represent the portion of the original substrate (sucrose) that remains unfermented. A single molecule of sucrose is composed of one glucose plus one fructose.

Glycogen is a storage carbohydrate in microbes. Microbes convert a portion of the sucrose to glycogen to use when the sucrose is depleted. Cattle can digest glycogen like they digest starch.

Fiber digestion was measured to determine how sucrose and the factors tested in these experiments could affect fermentation of a major component of forage.

Organic acids produced by fermentation in the rumen are a major energy source to the cow. Due to difficulties with our gas chromatograph, these assays will be run on HPLC in Dr. Paul Weimer's lab. He indicates that they should be ready by October.

Results: pH Study

Microbial Protein Yield

- ◆ Maximum microbial protein yield was recorded at hour 12 for the neutral medium and at hour 20 for the acid medium (Figure 1).
- ◆ Maximum microbial protein yield was almost double for the neutral medium compared to that of the acid medium, at 19.2 mg vs. 10.9 mg, respectively.
- ◆ The higher microbial crude protein yield of the neutral medium translated into a 77 % $[(0.16 - 0.09)/0.09]$ increase in efficiency compare to the acid medium (Table 1).

Table 1. Least squares means of microbial protein yield and efficiency.

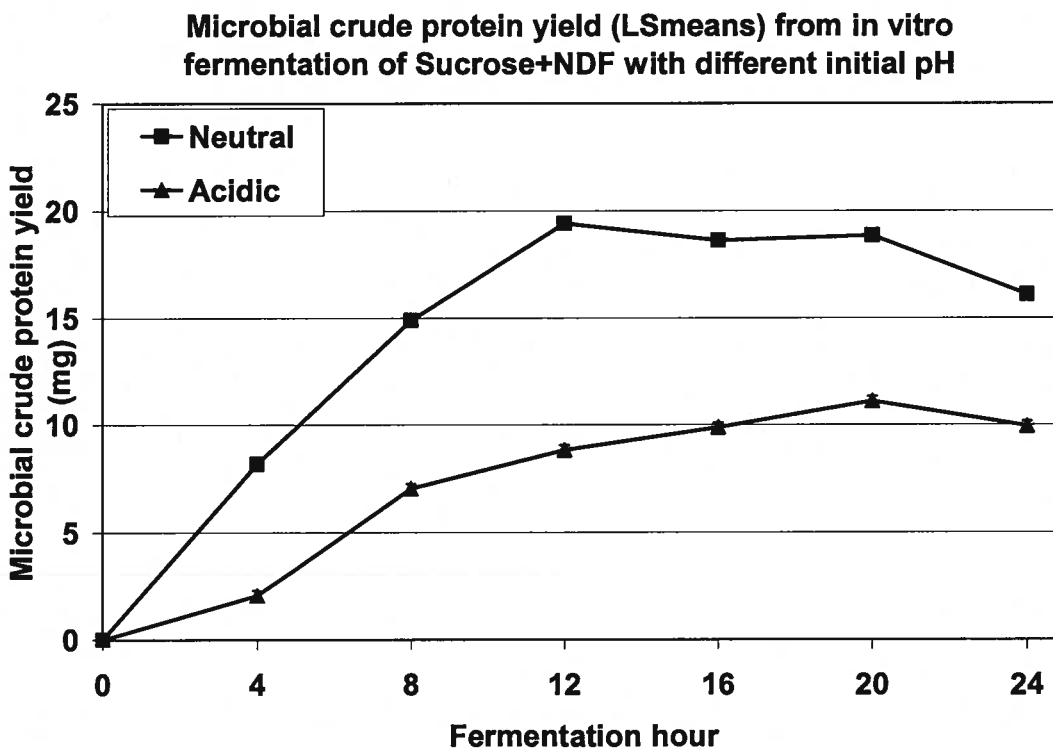
pH ¹	Maximum TCACP yield (mg)	SE ²	TCACP (mg) / Sucrose ³ (mg)
6.7	19.2	0.22	19.2/120 = 0.16
5.7	10.9	0.22	10.9/120 = 0.09

¹ Starting medium pH.

² Standard Error

³ Milligrams of sucrose per fermentation tube (120 mg)

Figure 1.



Sugars

- ◆ Sucrose rapidly disappeared from the fermentation medium, with only 69% or 40% of the original 120 mg found even at 0 hour in the neutral and acidic fermentations, respectively (Table 2 & Figure 2). The acidic medium contained less sucrose at the 0 hour than did the neutral medium ($P < 0.01$). The amount of sucrose equivalent [sucrose + 0.95 x (glucose+fructose)] remaining at hour 0, as a proportion of the initial 120 mg sucrose, was similar for the two media (acidic 81%; CA: 83 %; $P = 0.67$) (Table 2). By hour 4 no sucrose could be detected in either medium (Figure 2).
- ◆ Both glucose ($P < 0.01$) and fructose ($P < 0.01$) amounts were higher for the acidic medium at hours 0 and 4 (Figure 3 and 4). This could be due to a greater hydrolysis of sucrose and/or lower use at the more acidic pH.
- ◆ Glucose was no longer detected in the neutral medium at hour 4, or in the acidic medium at hour 8 (Figure 3).
- ◆ Fructose was no longer detected in either media at hour 8 (Figure 4).
- ◆ The increase in fructose for the acidic medium at hour 4 could indicate that upon hydrolysis of sucrose, glucose is the preferred energy source for microbes at least at that pH.

Table 2. Least squares means \pm standard error for glucose, fructose and sucrose remaining at hour 0 and 4, and total sucrose equivalent at hour 0.

Medium pH ¹	Glucose (mg)	Fructose (mg)	Sucrose (mg)	Total sucrose equivalent ² (mg)
<u>Hour 0</u> 6.7	8.41 \pm 0.18	7.09 \pm 0.94	82.86 \pm 0.83	97.59
5.7	26.51 \pm 0.18	26.91 \pm 0.94	48.28 \pm 0.83	99.03
<u>Hour 4</u> 6.7	-0.39 \pm 0.18	2.07 \pm 0.94	-0.30 \pm 0.83	
5.7	6.11 \pm 0.18	38.78 \pm 0.94	-0.25 \pm 0.83	

¹ Starting pH of fermentation medium.

² (Glucose + Fructose) x 0.95 + Sucrose = Total sucrose equivalent

Figure 2. Remaining sucrose (LSmeans) from in vitro fermentation of Sucrose+NDF with different initial pH

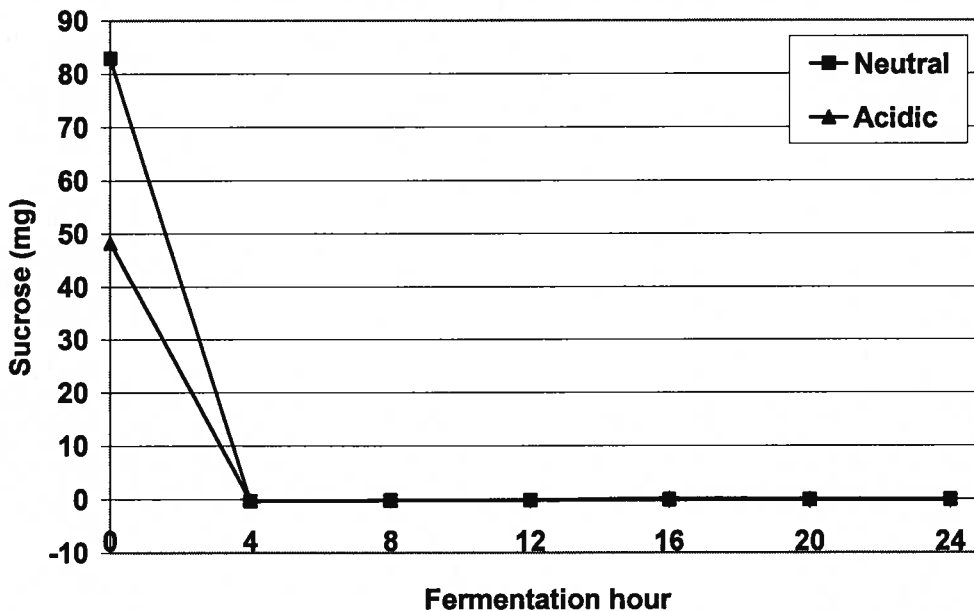


Figure 3.

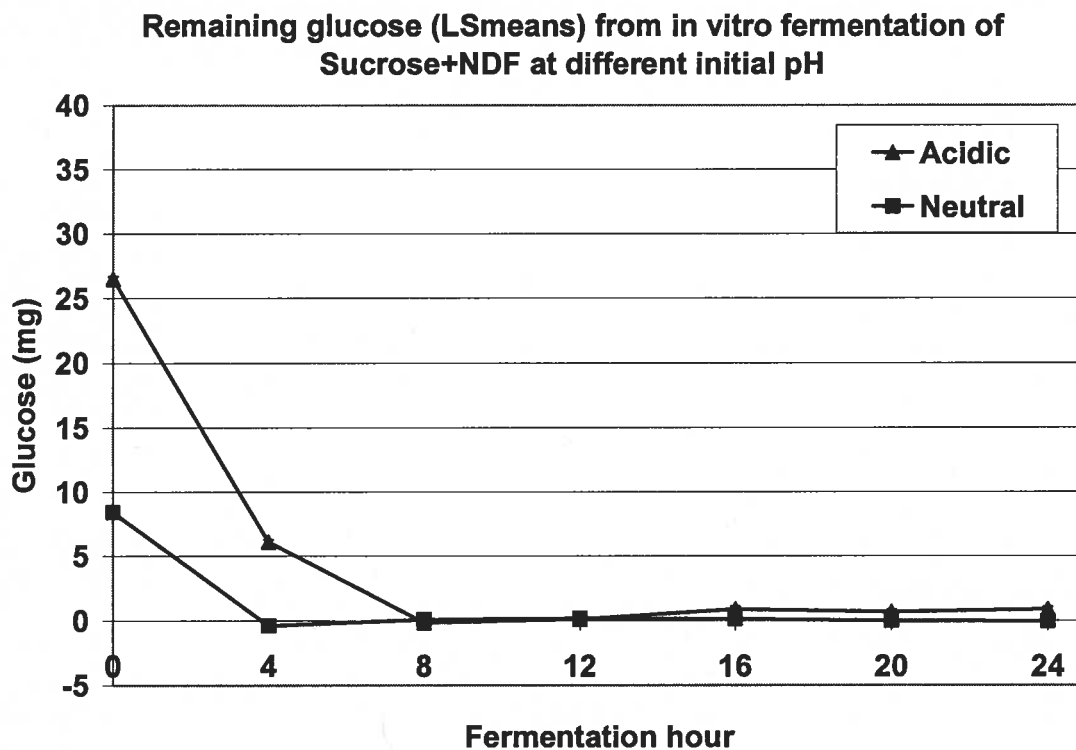
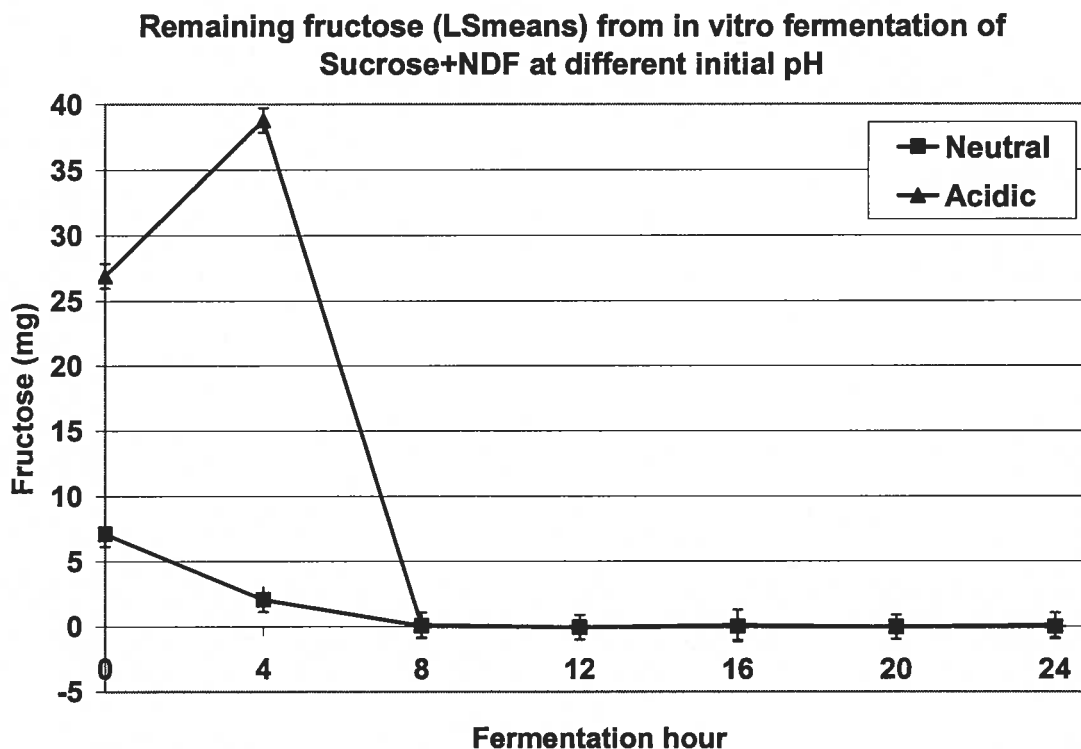


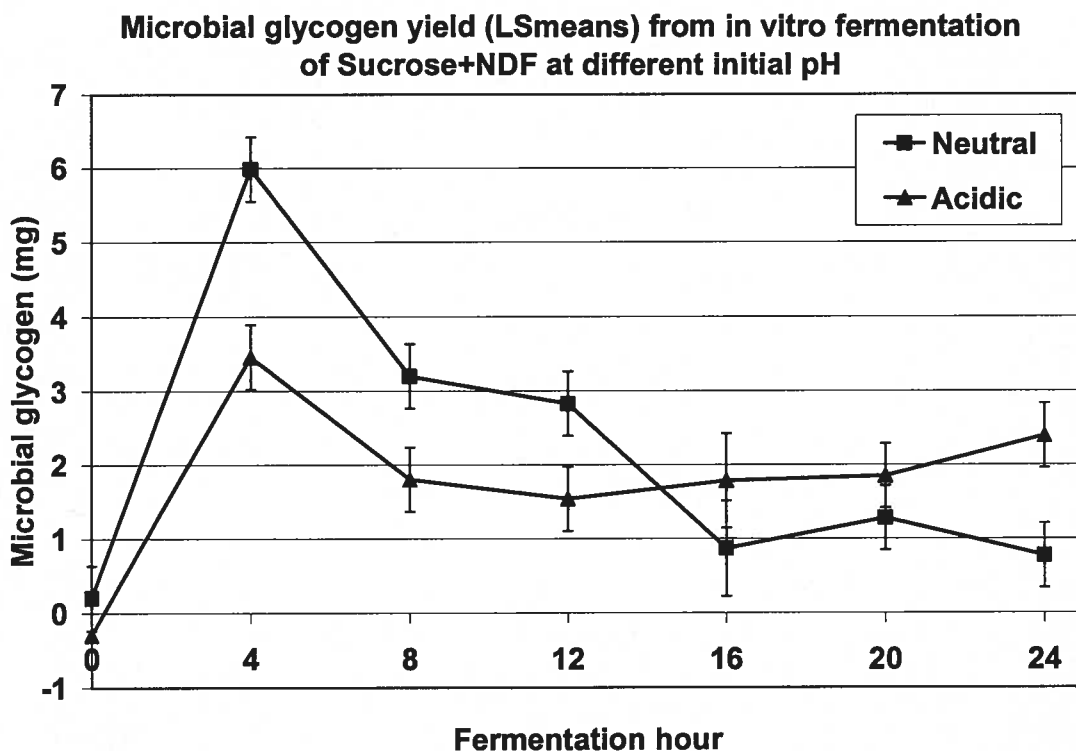
Figure 4.



Microbial Glycogen

- ◆ The maximum microbial glycogen yield for both media was recorded at hour 4, at 6.0 mg and 3.5 mg for the neutral and acidic media, respectively (Figure 5).
- ◆ At a more acidic starting pH, sucrose-utilizing microbes converted less sucrose to glycogen by 4 hours ($P = 0.04$), but appeared to utilize less over the following 20 hours than did microbes that had a more neutral starting pH.
- ◆ The efficiency of yield of glycogen per unit of sucrose was lower for the acidic than for the neutral medium (0.029 and 0.050 mg glycogen/mg of sucrose, respectively; $P = 0.04$).

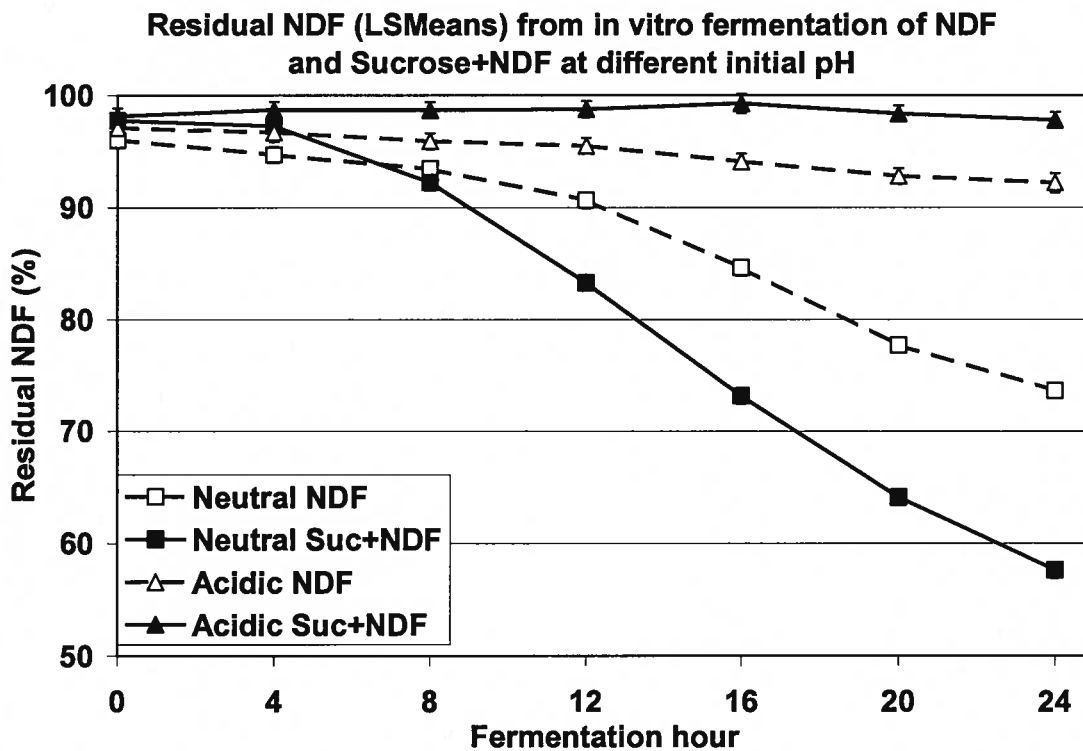
Figure 5.



Fiber (NDF) Digestion

- ◆ Neutral detergent fiber digestibility differed between the two media ($P < 0.01$) as well as between NDF alone or NDF+Sucrose within each medium ($P < 0.01$) (Figure 6).
- ◆ Neutral detergent fiber digestibility was decreased for both SuNDF and iNDF at the more acidic pH ($P < 0.01$).
- ◆ For the neutral medium, 24 hour NDF digestibility was increased when sucrose was present, with NDF digestibility of Sucrose+NDF at 42.4%, and at 26.4% for NDF alone ($P < 0.01$).
- ◆ The reverse was true for the acidic medium, where 24 hour NDF digestibility for Sucrose+NDF was 2.2% and for NDF alone was 7.8% ($P < 0.01$).

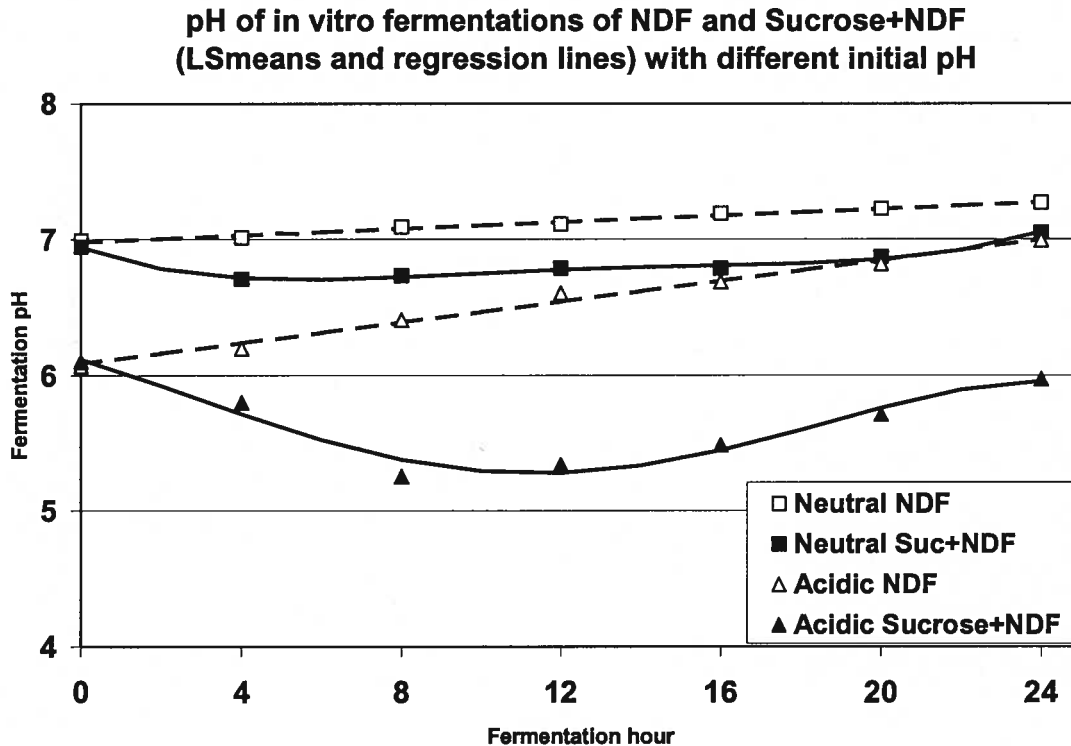
Figure 6.



pH

- ◆ Fermentation pH increased linearly for NDF fermented neutral or acidic media (neutral: $r^2 = 0.54$; acidic $r^2 = 0.92$), and decreased quadratically in fermentations of Sucrose+NDF (neutral $r^2 = 0.72$; acidic: $r^2 = 0.83$) for both media (Figure 7).
- ◆ At their lowest point, the difference in pH between NDF and Sucrose+NDF fermentations was almost four (3.74) times greater for the acidic medium (-1.16 pH units at hour 8) compared to the neutral medium (-0.31 pH units at hour 4) ($P < 0.01$).
- ◆ The pH of the acidic medium remained below 6 for the duration of the fermentation.
- ◆ Further interpretation of these results will wait until we have the results on the organic acid production. There is the possibility that addition of citric acid to decrease the pH of the acidic medium reduced the buffering capacity of that medium, so that its pH would change more with each addition of acid (P. Doane, personal communication).

Figure 7.



Summary of pH Study

In this in study:

- ◆ Microbial crude protein yield from sucrose was greater and peaked sooner at the more neutral starting pH than at the more acidic starting pH.
- ◆ As compared to fermentation of NDF alone, NDF digestion was increased when sucrose was added and starting pH was neutral.
- ◆ NDF digestion at a more acidic pH was decreased whether sucrose was present or not, but was further depressed by sucrose addition. This affect may be related to the effect of sucrose on medium pH when initial pH is low.
- ◆ Microbial glycogen yield was also lower for the acidic medium and appeared to be utilized by the microbes to a lesser extent than for the neutral medium. The more rapid utilization of glycogen in the neutral medium could be due to a more rapid production of microbial crude protein (microbial growth) and thus a greater need for energy.
- ◆ Sucrose was rapidly degraded in both media, but glucose and fructose remained through hour 8 in the acidic medium. Once again this may be due to the higher rate of microbial activity in the neutral medium at the earlier fermentation hours as indicated by higher microbial protein and glycogen yield.
- ◆ It will be interesting to see if the lower efficiency of microbial protein and glycogen yields will be counterbalanced by the organic acid production in the acidic medium.

So What?

- ◆ We know that pH affects fiber fermentation, but it also appears to affect the yield of nutrients from sucrose fermentation.
- ◆ When feeding rations containing sucrose, the ruminal pH you start at may affect the amounts of nutrients supplied to the animal. At a lower pH the yield of microbial protein and glycogen are decreased.
- ◆ At a more neutral pH, probably when protein is not limiting, sucrose may act to increase fiber digestion, likely increasing nutrient supply to the animal.

Results: Protein Source Study

Microbial Protein Yield

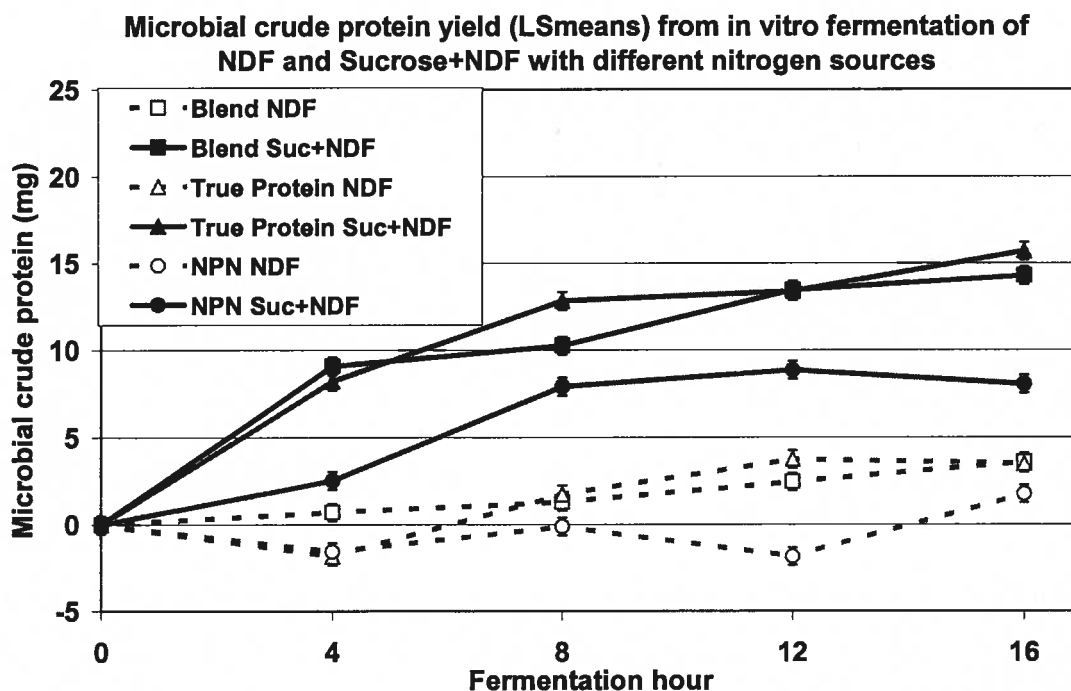
- ◆ The yield of microbial crude protein was lowest for fermentations containing only NonProtein Nitrogen (NPN)(Figure 8), whether NDF alone ($P = 0.01$) or sucrose+NDF ($P < 0.01$) were fermented, as compared to those containing true protein alone or a blend of true protein +NPN.
- ◆ At 16 h of fermentation, the microbial crude protein yield was greater for the true protein alone, as compared to true protein + NPN (Blend)($P = 0.02$) with Sucrose+NDF as substrate, but for NDF alone as substrate they did not differ ($P = 0.86$).
- ◆ With sucrose + NDF as a substrate, microbes provided with a source of true protein either as true protein alone or in a blend with NPN had nearly twice the efficiency of microbial protein yield per milligram of sucrose as did the microbes receiving NPN alone ($P < 0.01$) (Table 3). Microbes receiving true protein were more efficient than those receiving NPN+true protein ($P = 0.02$).

Table 3. Microbial protein yield at 16 hours of fermentation from NDF and NDF+sucrose with different nitrogen sources (least squares means).

Nitrogen Source	NDF alone	Sucrose+NDF	TCACP mg/Sucrose mg
NonProtein N	1.77	8.05	0.067
Blend	3.61	14.28	0.119
True Protein	3.50	15.70	0.131

Standard error for all values = 0.36 mg

Figure 8.



Sugars

- ◆ At hour 0 more sucrose was detected in media containing true protein or a nitrogen source blend compared to the medium containing only nonprotein nitrogen (NPN) ($P < 0.01$). However, glucose ($P < 0.01$) and fructose ($P < 0.01$) amounts were higher for the NPN medium (Table 4).
- ◆ The percentage of sucrose equivalent remaining at hour 0, as a proportion of the initial 120 mg sucrose, was similar for the blend and true protein media (73.84 % and 77.27 %, respectively; $P = 0.62$), but NPN medium was much lower than the other two media (50.92%; $P < 0.01$) (Table 4).
- ◆ By hour 4 no sucrose was detected in any of the media (Figure 9).
- ◆ Glucose was not detected in either true protein-containing media at hour 4, and in the NPN-containing media by hour 8 (Figure 10).
- ◆ By hour 4 no fructose was detected for NPN+true protein medium. In comparison, the NPN medium had a high level of fructose and the medium containing only true protein an intermediate level (Figure 11). The NPN medium differed from the true protein-containing media ($P < 0.01$), which differed from each other ($P < 0.01$). No fructose was detected by hour 8 in any media.

Table 4. Least squares means \pm standard error for glucose, fructose and sucrose remaining at hours 0 and 4, and total sucrose equivalent detected at hour 0.

Medium ¹	Glucose (mg)	Fructose (mg)	Sucrose (mg)	Total sucrose equivalent ² (mg)
<u>Hour 0</u> B	0.30 \pm 0.13	1.20 \pm 0.22	87.18 \pm 2.50	88.61
C	0.69 \pm 0.13	1.73 \pm 0.22	90.43 \pm 2.50	92.73
U	2.69 \pm 0.13	2.34 \pm 0.22	56.32 \pm 2.50	61.10
<u>Hour 4</u> B	-0.47 \pm 0.13	0.01 \pm 0.22	-0.34 \pm 2.50	
C	-0.72 \pm 0.13	5.87 \pm 0.22	-0.48 \pm 2.50	
U	3.13 \pm 0.13	13.91 \pm 0.22	0.076 \pm 2.50	

¹ B = True protein + Non protein nitrogen; C = All true protein; U = Non protein nitrogen

² (Glucose + Fructose) x 0.95 + Sucrose = Total sucrose equivalent

Figure 9.

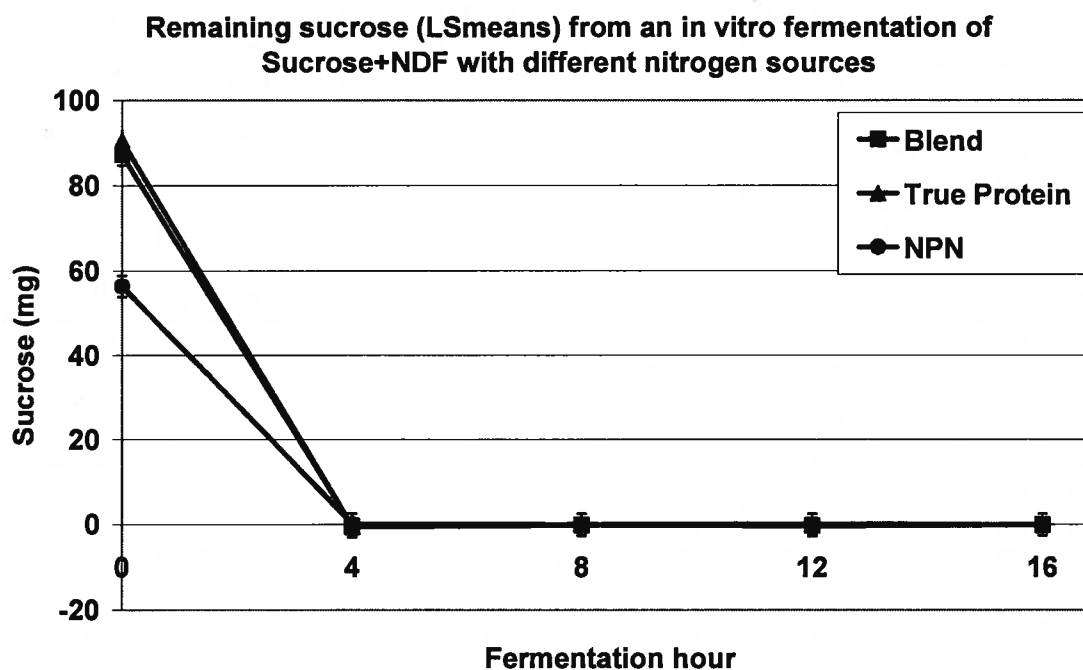


Figure 10.

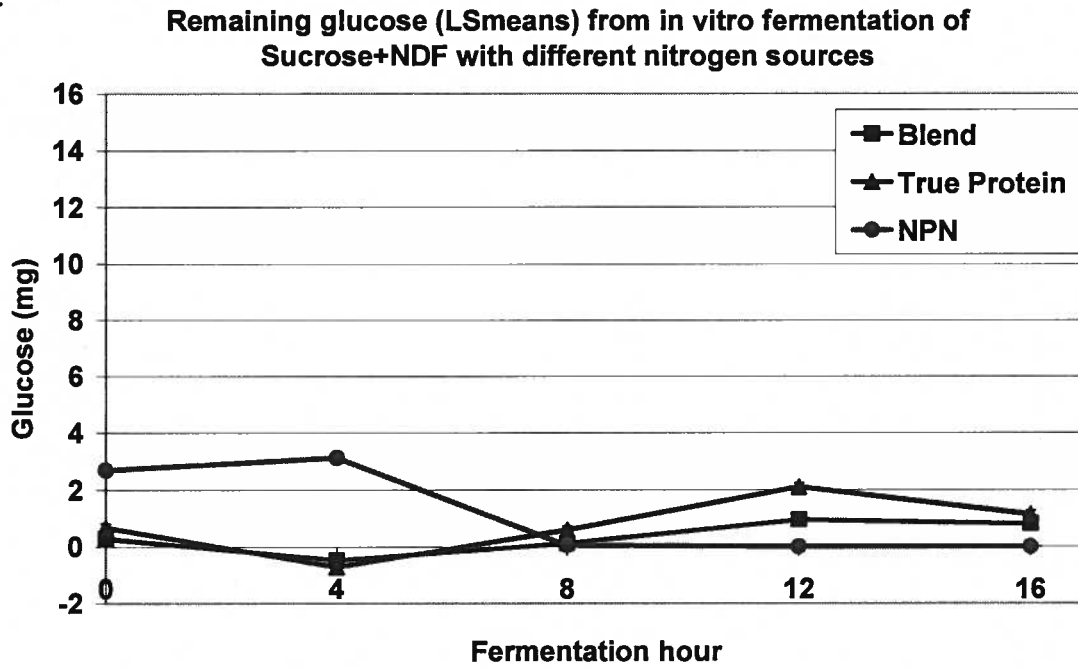
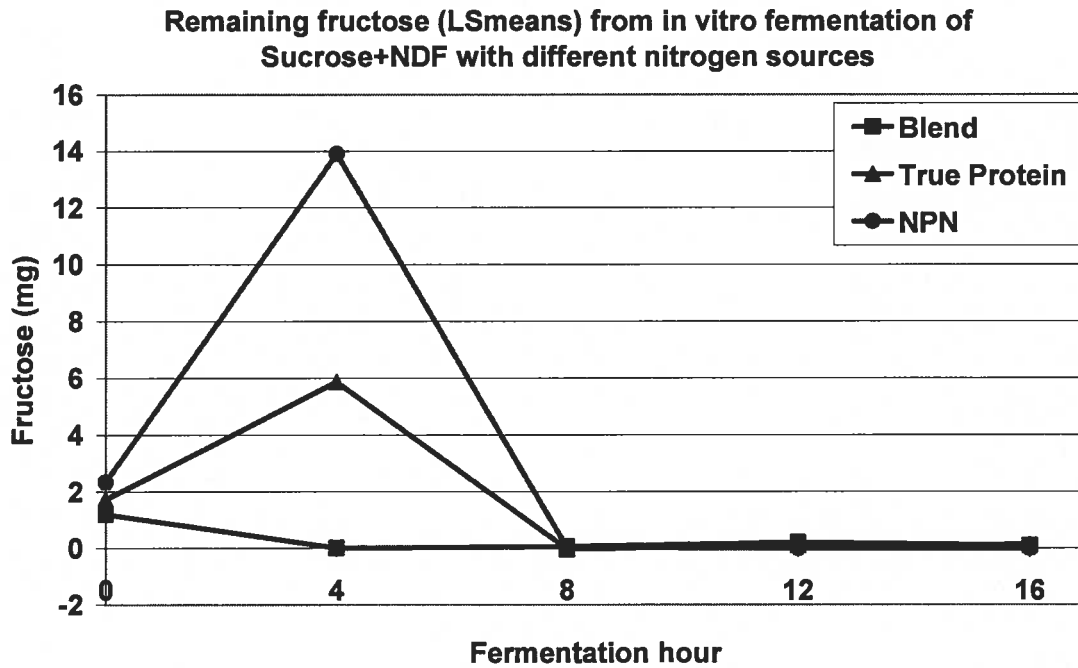


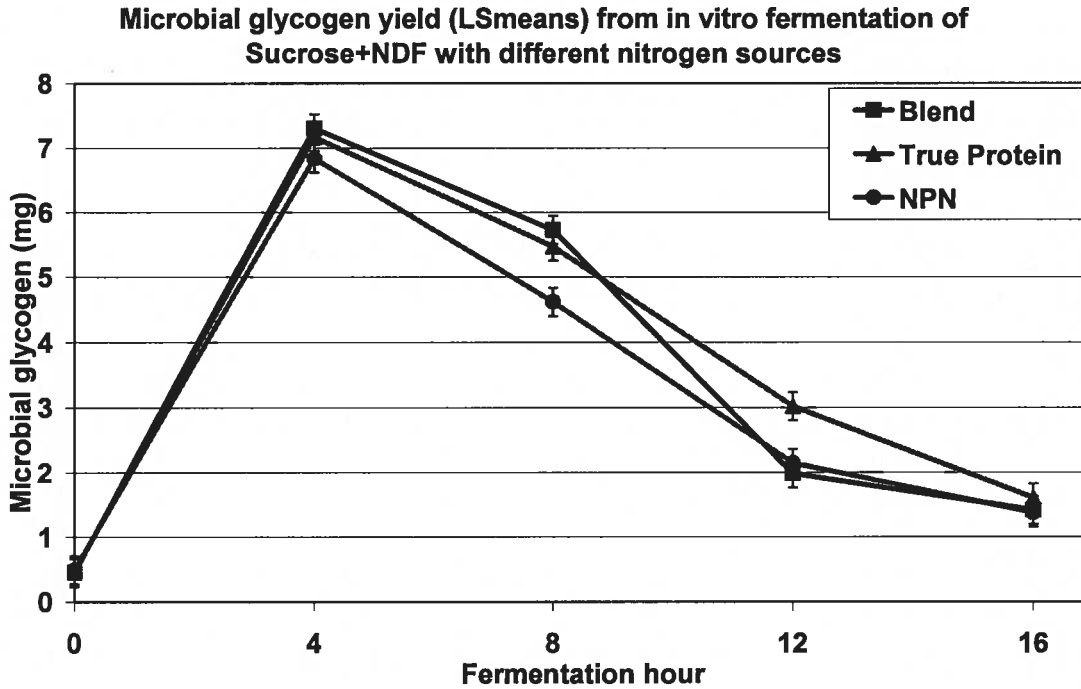
Figure 11.



Microbial Glycogen

- ◆ As in the pH study the maximum glycogen accumulation was at hour 4, with a steady decline thereafter (Figure 12).
- ◆ There was no detectable difference in maximal glycogen accumulation among nitrogen sources ($P = 0.64$). The average value for glycogen mg / sucrose mg = 0.059.
- ◆ Over the entire 16 hour fermentation, microbes provided only with NPN as a protein source had a lower content of glycogen ($P < 0.01$) than the treatments containing true protein which did not differ ($P = 0.80$). Glycogen content did not differ among nitrogen sources at 16 hours ($P = 0.72$).

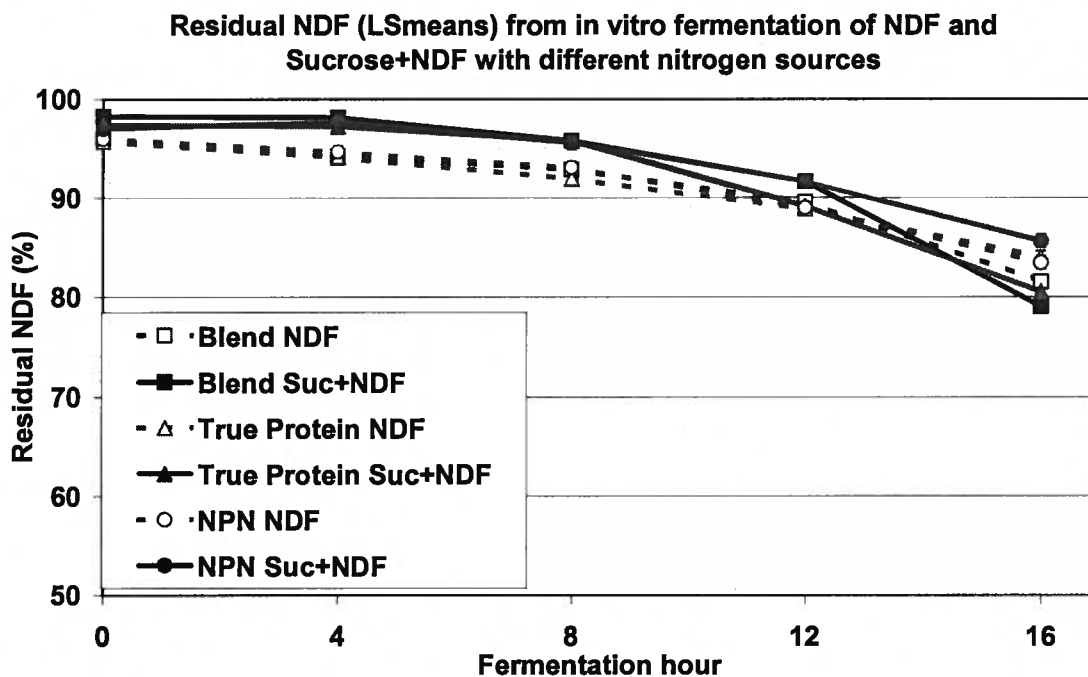
Figure 12.



Fiber (NDF) Digestion

- ◆ At hour 16 of the fermentation, nitrogen source for the microbes did not affect digestibility of NDF fermented alone ($P = 0.18$) (Figure 13).
- ◆ However, for the sucrose + NDF fermentations, the digestibility of NDF was lower ($P < 0.01$) for fermentations containing only NPN, than for those containing all true protein, or true protein + NPN, which did not differ from each other ($P = 0.18$).

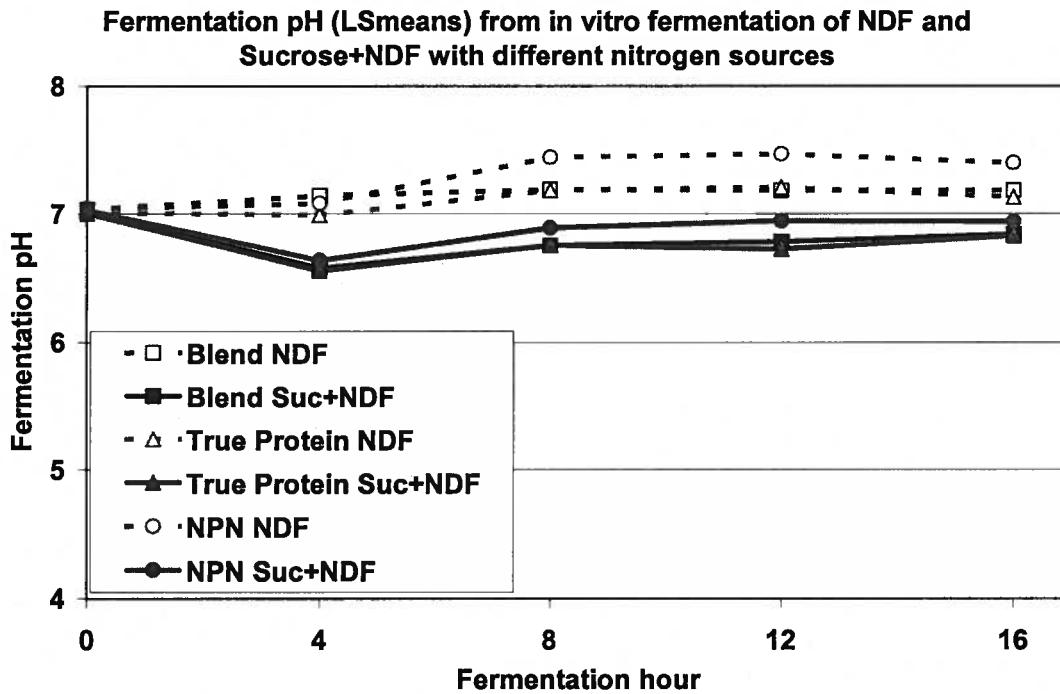
Figure 13



pH

- ◆ The NPN-only fermentations had a greater pH than did the true protein or true protein + NPN fermentations (+0.24 pH units for NDF and +0.11 pH units for sucrose+NDF fermentations) at 16 hours ($P < 0.01$). That may be a result of the added urea in the NPN fermentation hydrolyzing to release ammonia which is basic and can increase the pH.
- ◆ The pH followed the same pattern over the 16 hour fermentation for all three media, but was lower ($P < 0.01$) with Sucrose+NDF as compared to NDF alone (Figure 14).

Figure 14.



Summary of Protein Source Study

In this in study:

- ◆ Providing nonprotein nitrogen alone as a nitrogen source gave a 77% lower microbial protein yield and at hour 16 than if some true protein was provided. True protein alone gave a 9.9% higher yield of microbial protein than the true protein + NPN medium.
- ◆ Patterns of sucrose, glucose and fructose disappearance (use?) differed among nitrogen sources.
- ◆ Maximum microbial glycogen did not differ among nitrogen sources. Although they reached the same level by 16 hours, NPN alone had a lower glycogen value through most of the fermentation.
- ◆ When sucrose + NDF was fermented, the NDF digestibility at 16 hours was increased for incubations with true protein, as compared to NPN alone. The 16 hour NDF digestibility was not affected by nitrogen source when NDF was fermented alone.
- ◆ pH was highest on fermentations containing only NPN (mostly coming from urea).
- ◆ Addition of sucrose decreased fermentation pH as compared to fermenting only NDF.

So What?

- ◆ Adding true protein to diets and not nonprotein nitrogen alone may increase the yield of microbial protein when a sucrose source is fed.
- ◆ Inclusion of some true protein may also enhance fiber digestion when a sucrose source is fed.