# Alcomp Fermenter Studies

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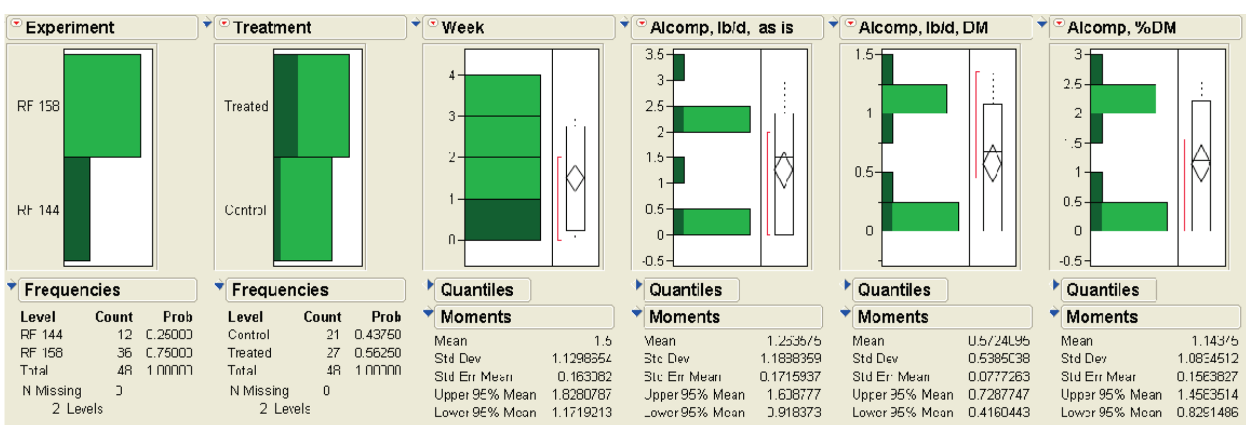
## Introduction

The focus of this report will be on combining of 2 fermenter studies and an addendum on amino acid flow from the second study. The combination of these studies will be an attempt to identify the important factors that control the responses from Alcomp. This information then can hopefully be used to provide a basis for conducting meaningful dairy studies and second will provide guidelines for the nutritionists in the field in the use of Alcomp

## Background of the Studies

Two studies were conducted. Below are frequency graphs depicting the setup of the two experiments. These plots are called whisker plots. Each of the bars is the frequency with which the determined number occurs. The line through the means diamond (95th confidence interval) is the median. The box represents the interquartile range at the 25th and 75th Percentile. The whiskers outside of the box represents upper or lower quartile + or – 1.5*(interquartile range). Dots outside of the whiskers indicate possible outliers. The red bar represents the densest 50% of the data. There is a lot of information represented in one of these plots. With large datasets, the frequency distribution begins to take on a normal bell shape look.

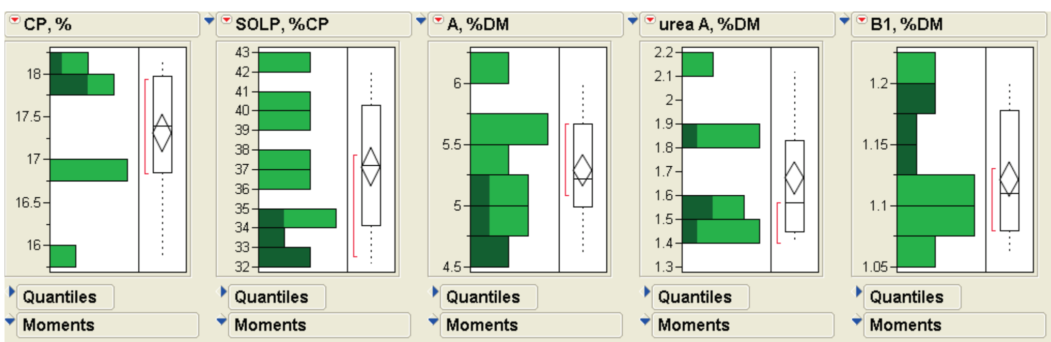
The dark green highlights the first experiment, RF 144. This first experiment had a control and 3 treatment levels of 1, 2, and 3lbs of Alcomp. The results from the first study allowed a selection of a 2lb inclusion level, varying the % forage in the ration over time.



Combined there were 48 observations across the two studies, with the second study, RF 158, having the greater number of observations (36).

CPM was used to do the final balancing of the rations. The initial balances were done by Stephen Mehen. The rations used in the fermenter studies were evaluated in CPM and then certain variables were placed into JMP (statistical software from SAS). With the inputs from CPM and the results from the studies, it will be possible to examine the nutritional inputs that are used to formulate rations so that relationships can be developed to provide guidelines for the field and for the design of future experiments.

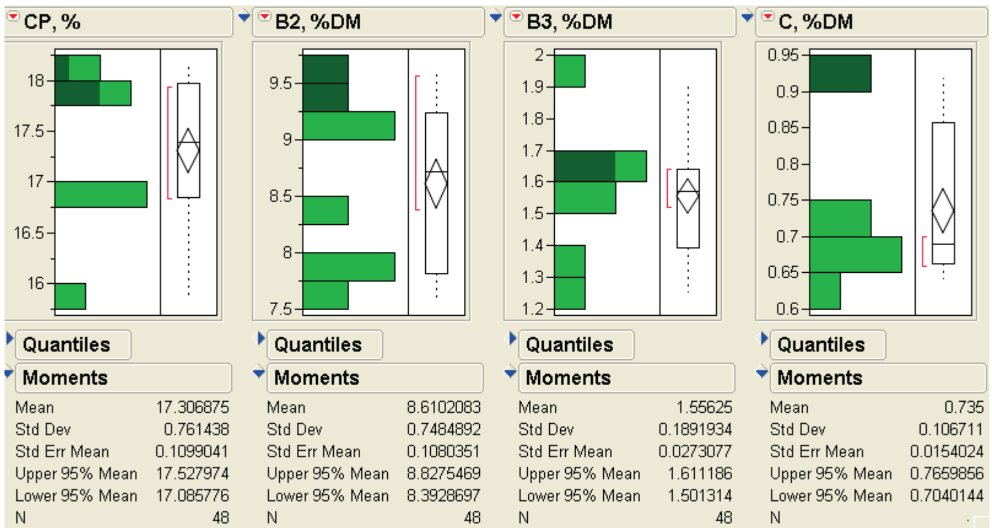
Below is the range of the protein fractions in the soluble protein. The dark green is again RF 144. The crude protein in the first experiment was high compared to the second experiment. Overall the crude protein in the combined experiments is 17.3%. The range in soluble protein, %CP, was wide across the two experiments, going from the low 30's to the low 40's, with a low soluble protein in the first experiment, as shown by the highlight which is very tightly clustered. The A fraction, a part of the soluble protein, is the rapidly degraded protein fraction. This, in CPM, includes the urea, ammonia, most of the peptides and other non protein nitrogen compounds. In CNCPS 6.1, the updated model appearing in the AMTS and NDS platforms, this value will only be $NH_3$ and urea. This will make the B1 pool much larger.




| | CP, % | SOLP, %CP | A, %DM | urea A, %DM | B1, %DM |
|---|---|---|---|---|---|
| Mean | 17.306875 | 37.11625 | 5.288125 | 1.671875 | 1.12125 |
| Std Dev | 0.761438 | 3.3467098 | 0.4440273 | 0.237976 | 0.0485809 |
| Std Err Mean | 0.1099041 | 0.483056 | 0.0640898 | 0.0343489 | 0.0070121 |
| Upper 95% Mean | 17.527974 | 38.088033 | 5.4170571 | 1.740976 | 1.1353564 |
| Lower 95% Mean | 17.085776 | 36.144467 | 5.1591929 | 1.602774 | 1.1071436 |
| N | 48 | 48 | 48 | 48 | 48 |

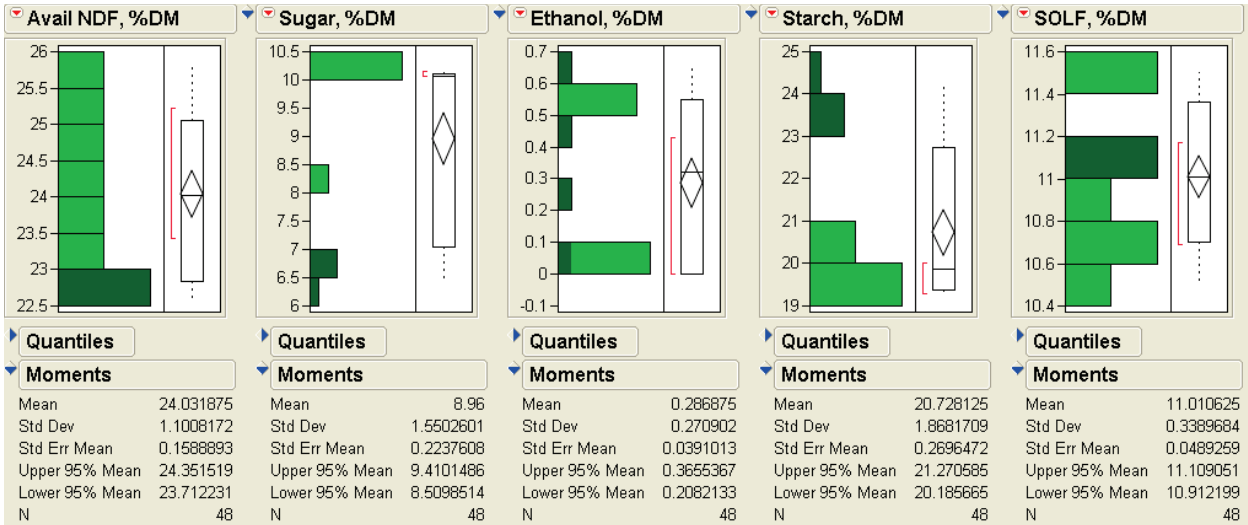
In the first experiment the A, as a %DM was low compared to the second experiment. It is of value to know the amount of the A fraction that is from urea. This urea will come from added urea and from the urea in Alcomp. Note that this is on the lower end of the distribution. The B1 is the other part of the soluble protein. This contains the true protein soluble in the borate phosphate buffer or in rumen fluid as well as large peptides. In CPM this protein fraction is mostly degraded in the rumen. In 6.1, 30% or greater escapes degradation in the rumen; this is a much bigger pool in 6.1. Because the A fraction was low in the first experiment, the B1 fraction was greater. The B1 fraction in 6.1 makes a significant contribution to meeting the MP requirements of the cow. At the same time because of the reduction of the RDP from the soluble protein fraction, it can become more important, when using Alcomp, to make sure that the RDP is adequate.

Below are the protein fractions in the insoluble protein. B2 is the protein fraction that is sensitive to the rate of passage because the rate of degradation of this fraction is close to the rate of passage. This protein is usually a higher quality protein. The B2 in the first experiment was quite high. This fraction contributes significantly to the RDP as well as the protein escaping fermentation in the rumen. In the first experiment, the B2 was high and fairly tight. The B3 fraction is lower quality and will mostly escape fermentation in the rumen. This protein will have a lower digestibility in the small intestine. This was intermediate in the first experiment. The C fraction is the estimate of the protein that is totally unavailable in the rumen and the rest of the intestine. This is the ADIP in feedstuffs. There was a range across the experiments and the in the first experiment the unavailable protein was the highest, although not that bad compares to many rations.

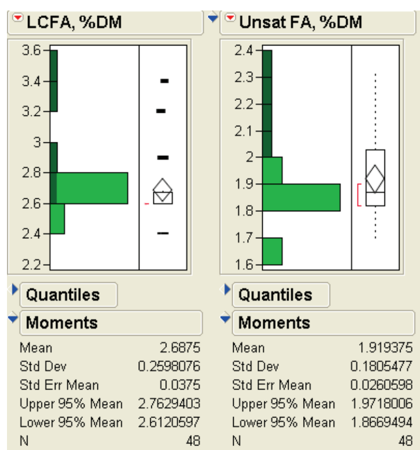



Below are the carbohydrate fractions. The amount of available NDF ranged from 22 to 26% with the first experiment being the lowest. The sugar content in the combined rations ranged from 6 to 10.5% with the first experiment having lower sugar content. Many times, we see sugar contents in rations in the Midwest and Northeast in the 3 to 5% range.

As would be expected the ethanol content of the rations in the first experiment would vary with the Alcomp inclusion level. It is interesting to note that on a DM basis the highest amount reached was only 0.7%. The starch levels were reasonable in the first experiment with the levels being lower in the second experiment. Actually quite a split between the two experiments. The lower starch is unusual for most rations; however in the second experiment higher forage rations was trying to be attained. The soluble fiber contents were quite high for these rations. This is reflective of the amount of alfalfa being included in the rations



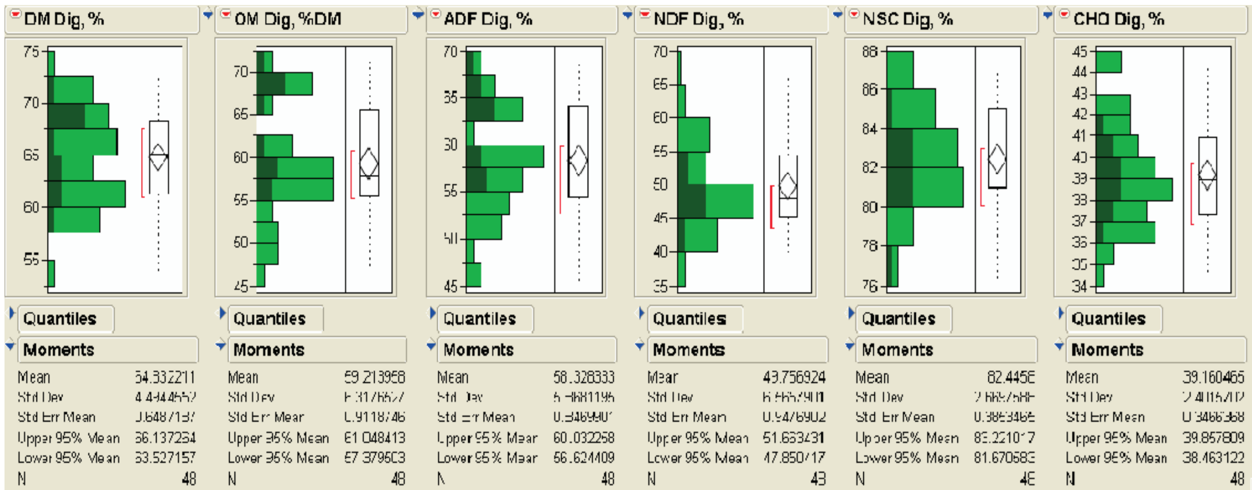

. We are now more sensitive to the fatty acid content of a ration. Below is the long chain fatty acids and the sum of the unsaturated fatty acids. Our concern has been centered on the unsat FA exceeding 2.6 %DM. The first experiment reached 2.4 %DM with a range overall from 1.6 to 2.4. Total Fatty Acids are now available from commercial forage labs and it has been noticed that the assumptions that we have been using for the TFA in feedstuffs are in many instances incorrect.



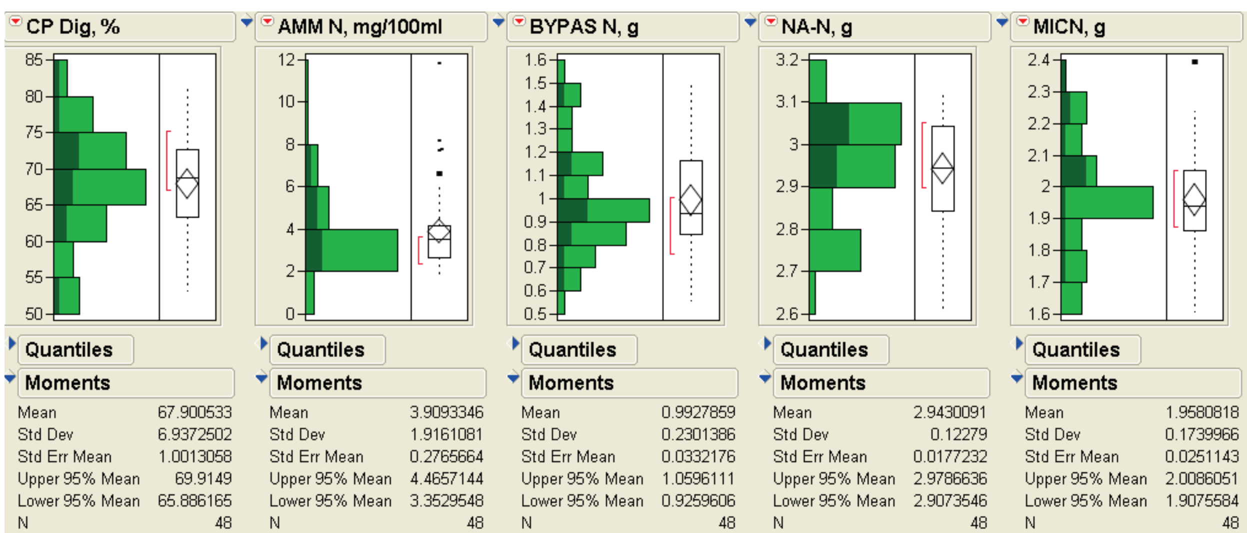

We do know that higher unsaturated fatty acids can negatively impact the digestibility of fiber. It seemed wise for the statistical analyses of these data to see if there was a negative impact from the unsaturated FA's.

**Fermenter Results**

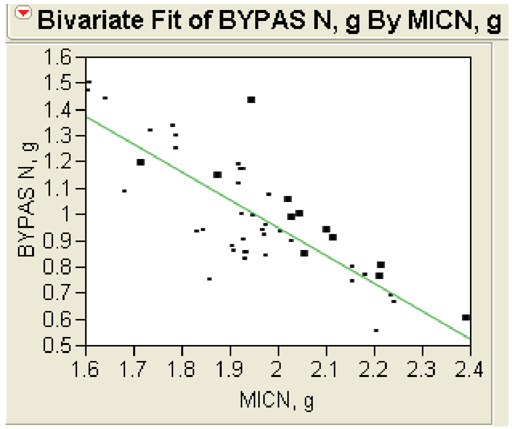
Below are the CHO digestibilities. There is quite a range in the digestibility of all of the CHO fractions. The one CHO fraction missing is the soluble fiber fraction.



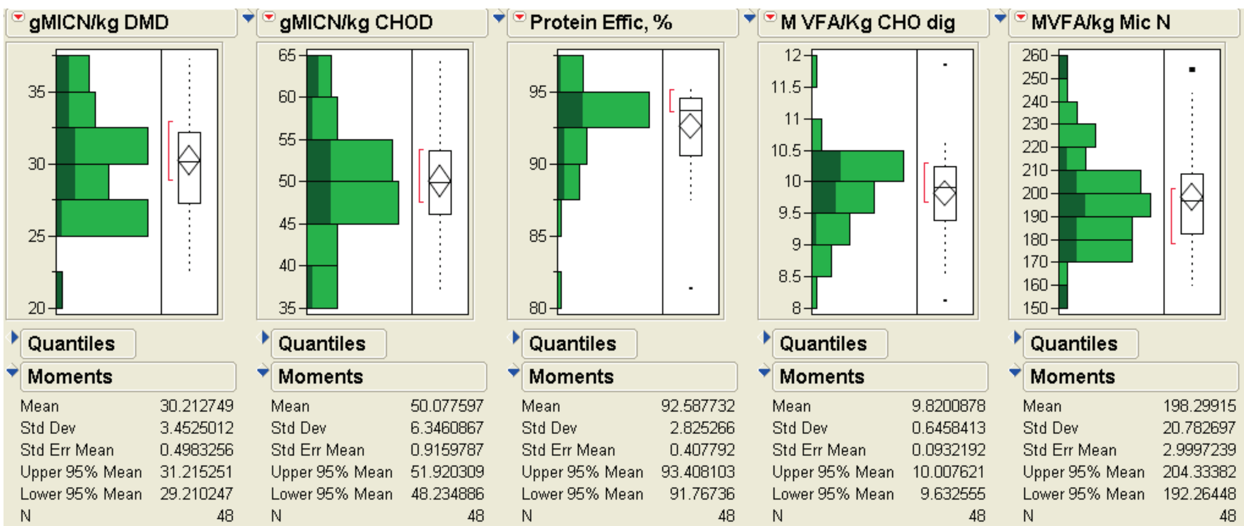

The results in the first experiment for DM, OM and ADF digestibility had a fairly large range. NDF, NSC (sugar + starch) and CHO digestibility had less of a range. The whiskers indicate that, except for the CHO Dig, the data are skewed.



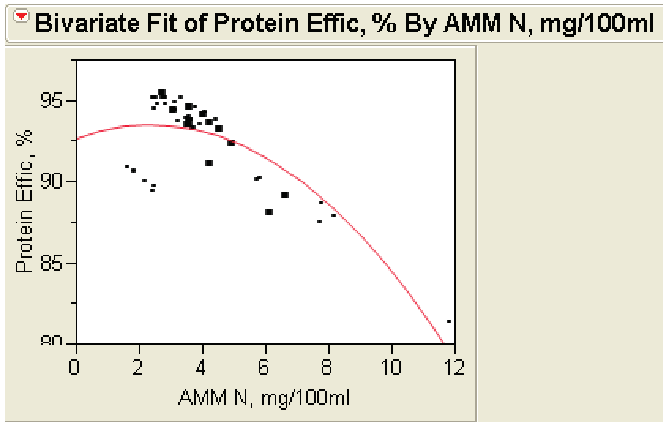

Above are the protein fractions. There is a wide range in the protein digestibility for the combined data and for the first experiment. The rumen $NH_3$ is very much skewed with some potential outliers. $NH_3$ levels below 4 to 5, could be considered limiting, leading to a poor fermentation. There is a large range in bypass N with a fairly large range in the first experiment. The NA-N represents the sum of the bypass N + the microbial N. As the microbial N increases the bypass N decreases (see figure below). In addition, the CP digestibility will also increases. There was a significant range in microbial N with the first experiment almost covering this range.




Below is the estimates of efficiency. With the exception of the gMICN/Kg CHOD, the data all represent some degree of being skewed. There is a wide range in microbial efficiency in the total dataset as well as in the first experiment. These results sometimes become hard to interpret.

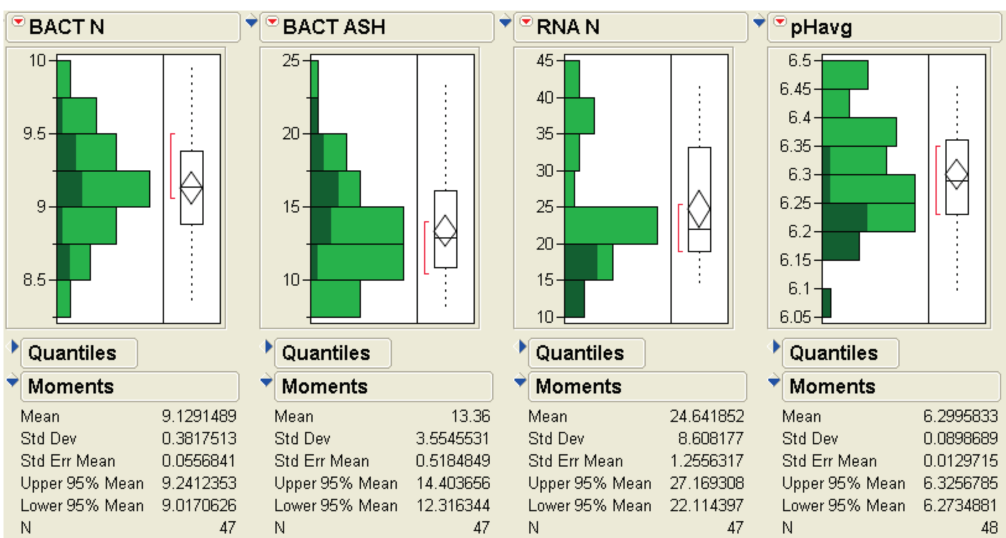



The best results are when you get an improvement in CHO digestion and also an increase in microbial yield. We can also get a reduction in CHO digestion with no change in microbial yield resulting in an increased microbial efficiency. This is not so good and this can happen frequently. The ability to measure Moles of VFA produced is a distinct advantage of the fermenters. It is possible to do this in a cow, but the cost is prohibitive, entailing extensive surgery. Moles of VFA/Kg CHO digestion show a fairly large range. As the amount of CHO digested increases it makes sense that the Moles of VFA will increase. However, if a product like Alcomp can improve the efficiency of the fermentation process in the rumen then the amount of VFA produced per kg of CHO fermented could be less, with more going into Microbial mass. The last efficiency calculation comes from the concept of Dr. Russell. This is moles of VFA produced per Kg microbial N yield. Reducing the VFA produced by bacteria from 260 moles to 150 moles is large. This means more of the CHO energy is going into microbial mass and less into waste products – VFA. The lower this number is the more coupled the fermentation. Generally, in the first experiment, with the exception of one outlier, the efficiencies and coupling were better than in the second experiment.

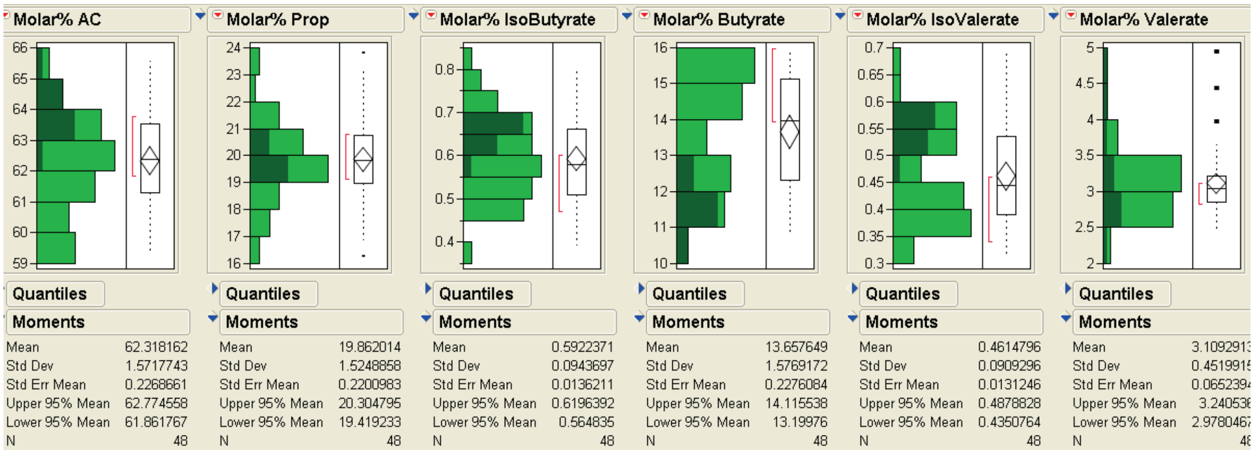



An improvement in protein efficiency usually means that there is not wasted N. This is depicted in the figure above. At low rumen $NH_3$, the utilization of N is highly efficient. However, at this low $NH_3$ there is an increased risk for impairment of CHO digestion.

Below is an attempt to identify changes in bacterial populations, looking at bacterial CP and RNA. The RNA-N in the first experiment was low compared to second experiment. The ruminal pH was low as well. Lower ruminal pH could cause a shift in the ecology.

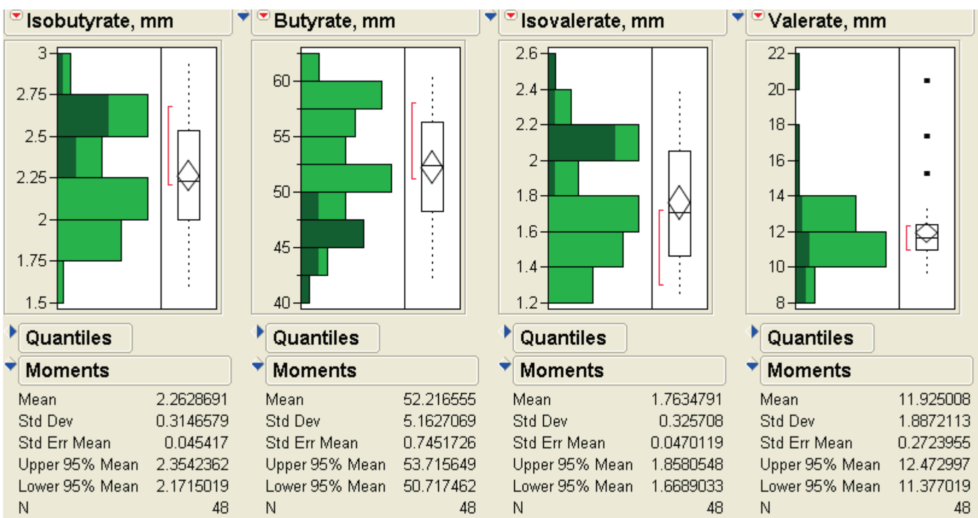



If we look above it will be noticed that the rations in the first experiment were higher in starch and also had a middle range in CHO digestibility. The combination of starch and fermentable fiber could have dropped the pH and had an impact on the ecology.

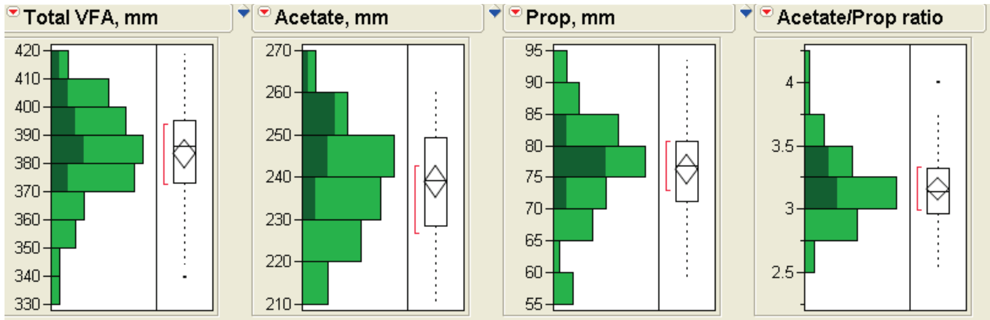



Above are the molar% VFA. This is each molar production divided by total Molar production. With the exception of Acetate, the ranges were large for each of the VFA. Acetate was high in the first experiment and butyrate was low. There were outliers in Prop and Valerate.

Below are the mmoles of VFA. Valerate in the first experiment was widely distributed with outliers. The rest were highly concentrated in one area.




Below are the total Mmoles of VFA, Acetate, Propionate and the AC/Prop ratios. The total VFA in the first experiment were higher. This probably explained, in part, the lower pH. Also on the second experiment, there was a higher proportion of forage in the ration.




Quantiles

Moments

| | Total VFA, mm | Acetate, mm | Prop, mm | Acetate/Prop ratio |
|---|---|---|---|---|
| Mean | 383.41463 | 238.96343 | 76.281826 | 3.1581571 |
| Std Dev | 17.353499 | 12.960714 | 8.0276504 | 0.289165 |
| Std Err Mean | 2.5047618 | 1.8707179 | 1.1586915 | 0.0417374 |
| Upper 95% Mean | 388.45356 | 242.72683 | 78.612813 | 3.2421219 |
| Lower 95% Mean | 378.3757 | 235.20003 | 73.95084 | 3.0741923 |
| N | 48 | 48 | 48 | 48 |

**Alcomp Relationships**

**Blend and analyses**

Several potential opportunities both from a marketing perspective and to help in the design if future studies will be examined with Alcomp. Alcomp is a blend of mainly distiller's solubles, ethanol, and urea.

| Ingredient | % | AF lb | Cum. lb | Nutrient | DM | AF |
|---|---|---|---|---|---|---|
| 23N liquid urea | 21.2500 | 425.0000 | 425.0000 | Dry Matter (%) | 100.00 | 45.33 |
| 10N - 34P - 0 | 0.5000 | 10.0000 | 435.0000 | Forage (%) | 0.00 | 0.00 |
| Ethanol | 11.5000 | 230.0000 | 665.0000 | Crude Prot (%) | 85.48 | 38.75 |
| Ethyl Acetate | 0.5000 | 10.0000 | 675.0000 | RUP (%CP) | 0.87 | 0.87 |
| CornDistSolubles | 66.2500 | 1325.0000 | 2000.0000 | RDP (%CP) | 99.13 | 99.13 |
| Totals | 100.0000 | 2000.0000 | | RDP (%) | 84.73 | 38.41 |
| | | | | Sol Prot (%CP) | 92.71 | 92.71 |
| | | | | ME (mCal/lb) | 1.34 | 0.61 |
| | | | | NEl (mCal/lb) | 0.87 | 0.39 |
| | | | | Nem (mCal/lb) | 0.87 | 0.39 |
| | | | | NEg (mCal/lb) | 0.61 | 0.28 |

The distiller's solubles come from an ethanol producer, who manufactures specifically for the medical industry which means that it is under a very strict manufacturing protocol. The resultant distiller's product will be consistent and high quality. As can be seen above the distiller's solubles makes up a significant % of the product and on a dry matter basis it is 49.1% DM.

Given that the solubles are having such an impact (ethanol is 24.9 %DM), it is important that a good representative analysis of this part of the blend be available. The product is a 32.7% protein product (CVAS analysis March 2010). There is a May analysis which is different and will not be used in this discussion. When we look at the detailed protein analysis, we see that the soluble protein (measured) is 61.3% CP. We assume that this soluble protein is 100% NPN (not measured). This is based on default numbers without a good basis for the CNCPS 5.0/CPM model.. A step forward was taken with CNCPS 6.1. It was decided that we needed a measured NPN. The NPN is now ammonia. This is a routine measurement at most laboratories. It is suggested that the $NH_3$ content of the solubles will be low. This results in a change in the fractionation of the soluble protein (A & B1) changing. The A fraction will decrease significantly and the B1 will now be a large number. It will be noted that when this

| Feed Name | Cost | DM | Date | |
|---|---|---|---|---|
| CornDistSolubles | 0.00 | 33.6000 | 00-00-0000 | Concentrate |

Protein | Carbohydrate | Fat | MinVit | Amino Acids | Quick Edit

| | Protein | %DM | %CP | %SP | Mineral | Concentration |
|---|---|---|---|---|---|---|
| | Crude Prot | 32.7000 | | | Ca | 0.0700 |
| | Soluble Prot | 20.0124 | 61.2000 | | P | 1.3000 |
| | NPN | 20.0124 | | 100.0000 | Mg | 0.6300 |
| | ADIP | 1.8000 | 5.5046 | | K | 2.0300 |
| | NDIP | 4.1000 | 12.5382 | | S | 1.4100 |
| | Carbohydrate | %DM | %NDF | | Na | 0.2770 |
| | ADF | 4.3000 | | | Cl | 0.3100 |
| | NDF | 8.6700 | | | Fe | 190.0000 |
| | peNDF | 0.0000 | 0.0000 | | Zn | 118.0000 |
| | Lignin | 0.9000 | 10.3806 | | Cu | 4.0000 |
| | Ash | 7.0000 | | | Mn | 24.0000 |
| | Ether Extract | 19.4000 | | | Se | 0.4000 |
| | NFC | 36.3300 | %NFC | | Co | 0.0000 |
| ChoA1 | Silage Acids | 0.0000 | 0.0000 | | I | 0.0000 |
| ChoA2 | Sugar | 16.7000 | 45.9675 | | Vitamin A | 0.0000 |
| ChoB1 | Starch | 6.4000 | 17.6163 | | Vitamin D | 0.0000 |
| ChoB2 | Soluble Fiber | 13.2300 | 36.4162 | | Vitamin E | 0.0000 |

becomes active in 6.1, the rate of digestibility will be around 35 to 40%. The soluble protein pool flows with the liquids out of the rumen in CNCPS 6.1. This translates into more of the protein in Alcomp contributing to the metabolizable protein, which will mean a reduction in the overall protein in the ration and will add value to Alcomp.

Protein | Carbohydrate | Fat | MinVit | Amino Acids | Quick Edit

| Nutrient Fraction | %DM | %CP | %SP | Rates | Intest Digest |
|---|---|---|---|---|---|
| Crude Prot | 32.7000 | | | | |
| Soluble Prot | 20.0124 | 61.2000 | | | |
| NPN | 20.0124 | | 100.0000 | | |
| ADIP | 1.8000 | 5.5046 | | | |
| NDIP | 4.1000 | 12.5382 | | | |
| | | | | %/h | %Escape |
| A | 20.0124 | 61.2000 | | 10000.0000 | 100.0000 |
| B1 | 0.0000 | 0.0000 | | 40.0000 | 100.0000 |
| B2 | 8.5876 | 26.2618 | | 6.0000 | 100.0000 |
| B3 | 2.3000 | 7.0336 | | 0.5000 | 100.0000 |
| C | 1.8000 | 5.5046 | | | |

This means that the amino acid content of the solubles becomes more important because they are now contributing to meeting the amino acid requirement of the cow. It should be added that the assumed AA content of the product looks inappropriate.

The carbohydrate fractions for the distiller's solubles are below. The fiber is not a large part of the product. This will mostly be corn bran residue which has an assumed reasonable rate of digestion of 7 %/h. It should be added that the available fiber comes from assuming the unavailable fiber is lignin*2.4, which might not be appropriate for this product. The product is 36.3% NFC. Most of this is sugar with some starch left and a significant amount of residual material we call soluble fiber. We need to define the nature of the sugars in the product. These assays will be available from CVAS in the next month. There is too much "soluble fiber" to ignore. There can be compounds in this fraction that are enhancing the fermentation in the rumen that we need to know about. With the HPLC capability at CVAS, we might be able to determine what these are.

Protein | Carbohydrate | Fat | MinVit | Amino Acids | Quick Edit

| | Nutrient Fraction | %DM | %NDF | Rates | Intest Digest |
|---|---|---|---|---|---|
| | | | | %/h | %Escape |
| | ADF | 4.3000 | | | |
| | NDF | 8.6700 | | | |
| | peNDF | 0.0000 | 0.0000 | | |
| | Lignin | 0.9000 | 10.3806 | | |
| ChoB3 | Avail NDF | 2.4100 | 27.7970 | 7.0000 | 20.0000 |
| ChoC | Unavail NDF | 2.1600 | 24.9135 | | |
| | Ash | 7.0000 | | | |
| | | | | | |
| | Ether Extract | 19.4000 | | | |
| | | | %NFC | | |
| | NFC | 36.3300 | | | |
| ChoA1 | Silage Acids | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| ChoA2 | Sugar | 16.7000 | 45.9675 | 40.0000 | 100.0000 |
| ChoB1 | Starch | 6.4000 | 17.6163 | 40.0000 | 98.0000 |
| ChoB2 | Soluble Fiber | 13.2300 | 36.4162 | 40.0000 | 80.0000 |

The fatty acid analysis is below. The ether extract is measured. The total fatty acids (TFA) are assumed both in amount and in the profile. CVAS now has the capability to do fatty acid (FA) analysis and it is suggested that this be done when new samples are submitted. The assumed FA profile is that for corn distillers. It will be similar but does need to be checked. There are two important points: First the lipolysis rate of 500%/h means that most of the fat will be changed to free FA and will be biohydrogenated. If you look at the fatty acid profile, 81% of the TFA is the unsaturated FA that we are concerned about relative to milk fat depression. Understand, however, that at the suggested inclusion levels of Alcomp this usually should not be a problem. Only about 5% of the unsaturated FA will come from Alcomp.

Protein | Carbohydrate | Fat | MinVit | Amino Acids | Quick Edit

| | Composition | | Rates | | |
|---|---|---|---|---|---|
| | % DM | | Lipolysis | 500.0000 | %/h |
| Ether Extract | 19.4000 | % EE | Adjust factor | 0.0000 | |
| Total Fatty Acid | 15.5200 | 80.0000 | LCFA Intestinal Digestibility | | |
| Glycerol | 1.7072 | | Rumen Free | Rumen Non-lipolysed | |
| Pigment | 2.1728 | % TFA | % Intestinal | % Intestinal | |
| C12:0 | 0.0466 | 0.3000 | 95.3900 | 95.3900 | |
| C14:0 | 0.0388 | 0.2500 | 75.0600 | 48.5800 | |
| C16:0 | 2.3280 | 15.0000 | 72.4800 | 72.4800 | |
| C16:1 | 0.0155 | 0.1000 | 64.0000 | 64.0000 | |
| C18:0 | 0.3880 | 2.5000 | 72.8000 | 72.8000 | |
| C18:1T | 0.0078 | 0.0500 | 78.5600 | 0.0000 | |
| C18:1C | 2.7936 | 18.0000 | 89.2500 | 66.9300 | |
| C18:2 | 8.5360 | 55.0000 | 83.0000 | 77.6200 | |
| C18:3 | 1.2416 | 8.0000 | 77.5500 | 77.5500 | |
| Other | 0.1242 | 0.8000 | 58.1700 | 58.7100 | |
| Fat Type | 1 | | | | |

The blend results in the analyses below. The negative ash is the result of the need to get to a mass balance of 100%, because of the inclusion of urea which is not really protein. The product does contribute some trace minerals including selenium. At some point, it probably would be advantageous to reconfirm the trace mineral analysis of the product. The silage acids are confusing. This is used as a place to put the ethanol. We use to have this in the sugar area.

| Feed Name | Cost | DM | Date | |
|---|---|---|---|---|
| Alcomp Dairy7 | 0.00 | 45.3325 | 03-12-2010 | Concentrate |

Protein | Carbohydrate | Fat | MinVit | Amino Acids | Quick Edit

| | Protein | %DM | %CP | %SP | Mineral | Concentration |
|---|---|---|---|---|---|---|
| | Crude Prot | 85.4782 | | | Ca | 0.0344 |
| | Soluble Prot | 79.2481 | 92.7115 | | P | 0.9946 |
| | NPN | 79.2481 | | 100.0000 | Mg | 0.3094 |
| | ADIP | 0.8839 | 1.0340 | | K | 0.9968 |
| | NDIP | 2.0133 | 2.3553 | | S | 0.6924 |
| | Carbohydrate | %DM | %NDF | | Na | 0.1360 |
| | ADF | 2.1115 | | | Cl | 0.1522 |
| | NDF | 4.2573 | | | Fe | 94.2536 |
| | peNDF | 0.0000 | 0.0000 | | Zn | 63.7997 |
| | Lignin | 0.4419 | 10.3806 | | Cu | 1.9642 |
| | Ash | -41.0294 | | | Mn | 11.7849 |
| | Ether Extract | 9.5261 | | | Se | 0.1964 |
| | NFC | 43.7811 | %NFC | | Co | 0.0000 |
| ChoA1 | Silage Acids | 25.5979 | 58.4680 | | I | 0.0000 |
| ChoA2 | Sugar | 8.5441 | 19.5154 | | Vitamin A | 0.0000 |
| ChoB1 | Starch | 3.1426 | 7.1781 | | Vitamin D | 0.0000 |
| ChoB2 | Soluble Fiber | 6.4965 | 14.8385 | | Vitamin E | 0.0000 |

Below are the protein fractions. Most of the protein is in the A fraction (92.7 %CP). Some of this will be shifted to the B1 fraction (10 to 12% of the CP) when we receive an $NH_3$ analysis on the solubles. This will improve the bypass by about 50 - 70g per day of MP. The B2, B3, and C pools are from analytical results for the soluble protein, NDIP and ADIP to generate the A, B and C fractions. The protein that escapes, it is assumed for the all fractions, except the C fraction will have a 100% digestibility. This will give an overall 89% digestibility of the bypass protein.

Protein | Carbohydrate | Fat | MinVit | Amino Acids | Quick Edit

| Nutrient Fraction | %DM | %CP | %SP | Rates | Intest Digest |
|---|---|---|---|---|---|
| Crude Prot | 85.4782 | | | | |
| Soluble Prot | 79.2481 | 92.7115 | | | |
| NPN | 79.2481 | | 100.0000 | | |
| ADIP | 0.8839 | 1.0340 | | | |
| NDIP | 2.0133 | 2.3553 | | | |
| | | | | %/h | %Escape |
| A | 79.2481 | 92.7115 | | 229.4613 | 99.0098 |
| B1 | 0.0000 | 0.0000 | | 210.8757 | 100.0000 |
| B2 | 4.2167 | 4.9331 | | 6.0000 | 100.0000 |
| B3 | 1.1294 | 1.3213 | | 0.5000 | 100.0000 |
| C | 0.8839 | 1.0341 | | | |

The assumed amino acid profile of the protein that escapes fermentation is below. This needs to be verified. If some of the germ of the protein is included in the solubles then the lysine will be much higher. This is really a prolamin protein amino acid profile and could be wrong. We will return to the AA question again with the discussion of the AA data in the second experiment.

Protein | Carbohydrate | Fat | MinVit | Amino Acids

| Amino Acid | Concentration (%RUP) |
|---|---|
| Met | 0.5840 |
| Lys | 1.0025 |
| Arg | 2.0196 |
| Thr | 1.5183 |
| Leu | 4.4139 |
| Ile | 1.3529 |
| Val | 2.5500 |
| His | 0.8857 |
| Phe | 2.0926 |
| Trp | 0.7981 |

The Carbohydrate fractions are below. Most of the CHO is in the NFC with 58% of it in the silage acids (25.6 %DM) which is the ethanol. This has a Kd of 40 %/h, which will translate into about 18 to 20% of it escaping fermentation and being absorbed. The Kd will need to be adjusted when Alcomp is being used in the 6.1 model. The starch is assumed to be about 82% fermented in the rumen. This can now be verified with a CVAS 7 hour invitro starch degradability measurement. We pointed out earlier that we need to verify what the soluble fiber is and what the sugars really are.

Protein | Carbohydrate | Fat | MinVit | Amino Acids | Quick Edit

| Nutrient Fraction | | %DM | %NDF | Rates | Intest Digest |
|---|---|---|---|---|---|
| | ADF | 2.1115 | | %/h | %Escape |
| | NDF | 4.2573 | | | |
| | peNDF | 0.0000 | 0.0000 | | |
| | Lignin | 0.4419 | 10.3806 | | |
| ChoB3 | Avail NDF | 1.1834 | 27.7969 | 7.0000 | 20.0000 |
| ChoC | Unavail NDF | 1.0606 | 24.9125 | | |
| | Ash | -41.0294 | | | |
| | | | | | |
| | Ether Extract | 9.5261 | | | |
| | | | %NFC | | |
| | NFC | 43.7811 | | | |
| ChoA1 | Silage Acids | 25.5979 | 58.4680 | 35.7510 | 89.4721 |
| ChoA2 | Sugar | 8.5441 | 19.5154 | 40.0000 | 100.0000 |
| ChoB1 | Starch | 3.1426 | 7.1781 | 40.0000 | 98.0000 |
| ChoB2 | Soluble Fiber | 6.4965 | 14.8385 | 40.0000 | 80.0000 |

The lipids are below and reflect the FA profile of the solubles. These have been discussed in some detail above and there is little need to discuss them here further. We do need to verify both the TFA and the FA profile for completeness. The technical nutritionists will be looking at the TFA and FA now and be asking questions.

Protein | Carbohydrate | Fat | MinVit | Amino Acids | Quick Edit

| | Composition | | Rates | | |
|---|---|---|---|---|---|
| | % DM | | Lipolysis | 500.0003 | %/h |
| Ether Extract | 9.5261 | % EE | Adjust factor | 0.0000 | |
| Total Fatty Acid | 7.6209 | 80.0000 | LCFA Intestinal Digestibility | | |
| Glycerol | 0.8383 | | Rumen Free | Rumen Non-lipolysed | |
| Pigment | 1.0669 | % TFA | % Intestinal | % Intestinal | |
| C12:0 | 0.0229 | 0.3000 | 95.3900 | 95.3900 | |
| C14:0 | 0.0191 | 0.2500 | 75.0600 | 48.5800 | |
| C16:0 | 1.1431 | 15.0000 | 72.4800 | 72.4800 | |
| C16:1 | 0.0076 | 0.1000 | 64.0000 | 64.0000 | |
| C18:0 | 0.1905 | 2.5000 | 72.8000 | 72.8000 | |
| C18:1T | 0.0038 | 0.0500 | 78.5600 | 0.0000 | |
| C18:1C | 1.3718 | 18.0000 | 89.2500 | 66.9300 | |
| C18:2 | 4.1915 | 55.0000 | 83.0000 | 77.6200 | |
| C18:3 | 0.6097 | 8.0000 | 77.5500 | 77.5500 | |
| Other | 0.0610 | 0.8000 | 58.1700 | 58.7100 | |
| Fat Type | 1 | | | | |

## Statistical Analyses

The combined data will be examined using both traditional multiple linear regression analyses and non linear analyses using the Neural Net platform. The focus in these analyses is to identify nutritional inputs that we traditionally use that will provide management guidance in the use of Alcomp as well as to identify some weaknesses in our understanding that will allow us to design future studies either with cows or with continuous culture.

## Neural Net - NDF Digestibility

### Fit History

| Nodes | Penalty | RSquare |
|---|---|---|
| 3 | 0.01 | 0.67857 |

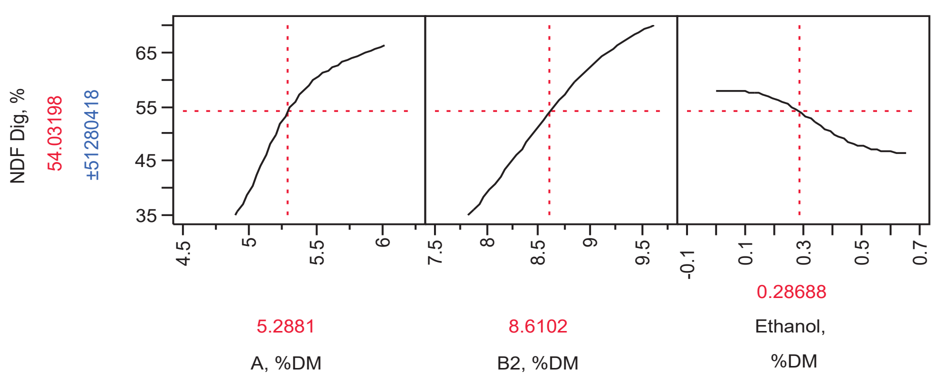
### Current Fit Results

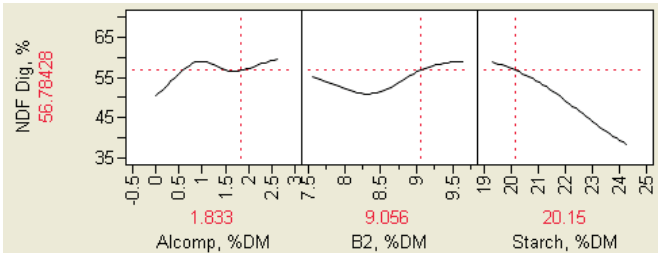
| | Objective |
|---|---|
| SSE | 15.107140596 |
| Penalty | 0.5511413122 |
| Total | 15.658281908 |
| N | 48 |
| Nparm | 16 |

16 Converged At Best
0 Converged Worse Than Best
0 Stuck on Flat
0 Failed to Improve
0 Reached Max Iter

| Y | SSE | RMSE | SSE Scaled | RMSE Scaled | RSquare |
|---|---|---|---|---|---|
| NDF Dig, % | 651.26279251 | 3.84726229 | 15.107140596 | 0.58595572 | 0.6786 |

### Prediction Profiler



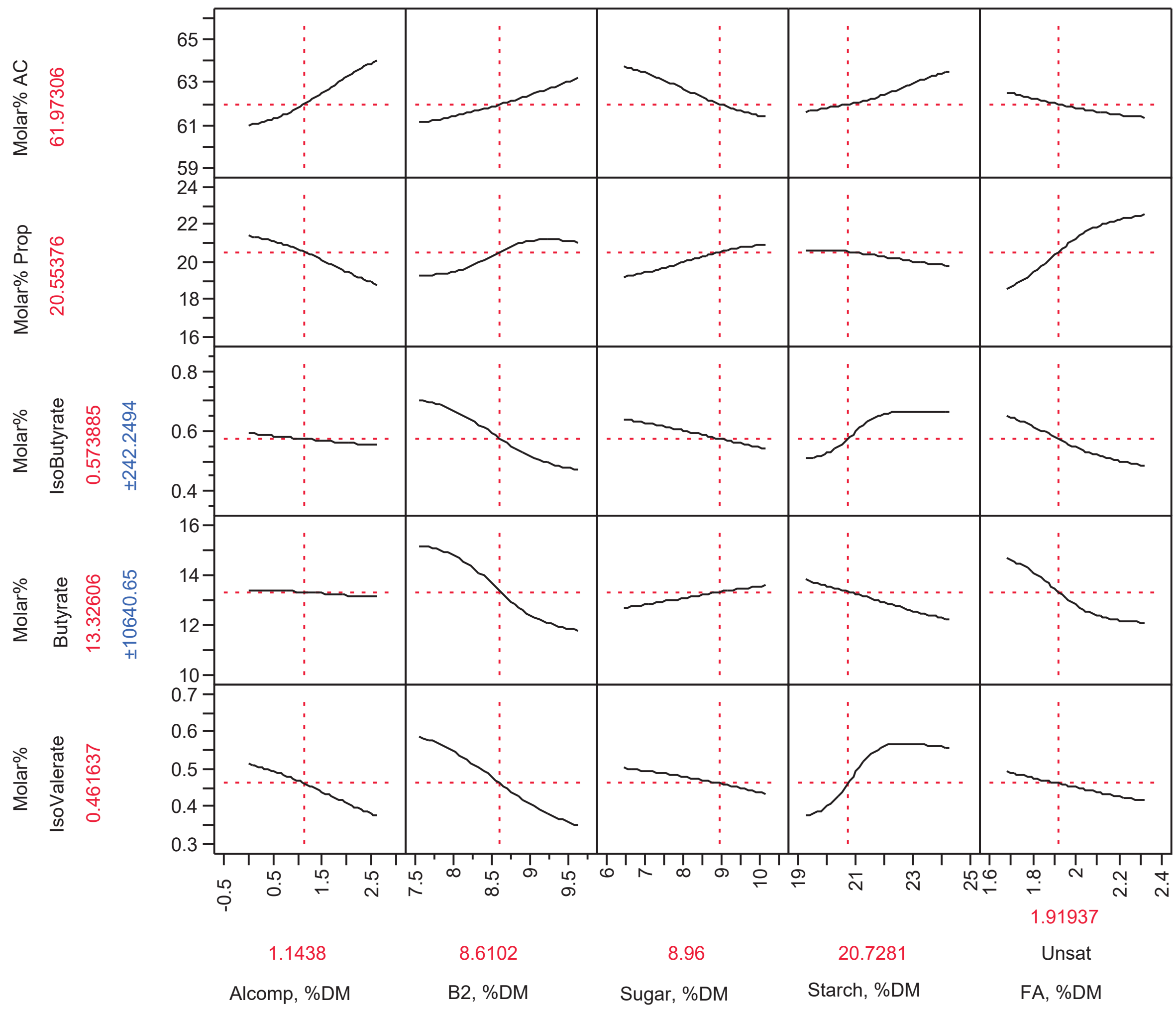



Above is an analysis of factors affecting the digestibility of the NDF. An analysis of many nutritional inputs were examined using multiple linear regression techniques. These 3 variables were selected as being the most significant. Alcomp was not initially included because it would have been difficult to separate out the factors within Alcomp affecting NDF digestibility. The A protein fraction is mostly urea with a NPN coming from other sources. It is obvious that this is important in enhancing fiber digestibility. The B2 fraction is rich in peptides which are a source of the isoacids that are critical to fiber digestibility. What is of interest and possibly needs more explanation is the non linear effect of the ethanol. Basically, this says that when you get much over 0.2 %DM, the ethanol has a negative effect on fiber digestibility. This equates to about 1 to 1.5 lbs of DM fed as Alcomp. This might serve not only as a guideline but also as an area that might need to be investigated further. An additional analysis was performed with Alcomp in the model. The starch and the B2 fraction were included. The A fraction was removed because Alcomp is a heavy contributor to this fraction. The model suggests that there is a response from Alcomp when it gets over 1% DM. The Alcomp was set at 1.8% DM, approximately at the level of a 1 lb DM inclusion level. Starch has a negative effect so this was kept low which might imply that higher forage rations will be beneficial. The B2 is on the high side which suggests that we need a good level of soybean meal in the ration. It is suggested that we can take away from this that Alcomp provides a positive response to NDF digestibility but this response is sensitive to ethanol and peptides levels as well as the amount of $NH_3$ which translates into urea. Part of the response from Alcomp is from the solubles and the mixture of peptides, sugars and the “soluble fiber” that is contributed by the product.

### Neural Net – Ruminal VFA, Molar%

| Y | SSE | RMSE | SSE Scaled | RMSE Scaled | RSquare |
|---|---|---|---|---|---|
| Molar% AC | 43.453884534 | 0.99377477 | 17.589288042 | 0.63226304 | 0.6258 |
| Molar% Prop | 40.796540003 | 0.96290918 | 17.544809741 | 0.63146312 | 0.6267 |
| Molar% IsoButyrate | 0.1175392268 | 0.05168507 | 13.198303613 | 0.54768736 | 0.7192 |
| Molar% Butyrate | 20.569773658 | 0.68373595 | 8.2720231025 | 0.43359027 | 0.8240 |
| Molar% IsoValerate | 0.0700142643 | 0.03989027 | 8.4679124001 | 0.43869415 | 0.8198 |

## Prediction Profiler




Above are the Molar% VFA. The beginning analysis started with examining the above model with traditional linear regression. The above X variables all had various levels of significance depending on the trait tested. I then went to the Neural Net platform above to see if there was an improvement in the R2's. There was. We need to look at each of the X variables (Alcomp, B2, etc) in the above graphical depiction as an independent variable which if varied relative to the rest of the variables in the model could, if there is an interaction with the other variables could change in the shape of the curve response. The way we test this is to move the vertical line either left or right to examine the response of the other variables, keeping them constant as we look at varying one variable. For example, let's say that we want to see the impact of moving Alcomp from it's current 1.14% that we show above up to 2% DM. What is the impact on the other X variables? The shape of the other curves does change. Equally important is to ask the question what happens to acetate and propionate of we move one of the nutritional inputs that we control up or down. For example if we move the unsaturated FA in the ration up what happens to the Acetate and propionate with Alcomp at a little over 1% DM. As the unsats move up the acetate declines modestly and the propionate increases dramatically; from these types of examinations we can potentially start to provide recommendations in the field as to where we need to formulate rations when feeding Alcomp. Also looking at these responses, we can use the improved insight as an approach for developing research protocols in the future.

We can see that the inclusion of Alcomp drives up acetate and decreases propionate as well as moving down isovalerate. The response of isobutyrate and butyrate was minimal. Valerate is not shown because interestingly both the linear and non linear models showed no prediction of change for Valerate.

Below is the ruminal VFA as production in mmoles/day. The Acetate/Propionate ratio is added. The overall $R^2$ of the model is lower than the VFA as molar%, however the overall significance for each of the variables with this model were significant in the linear model. The $R^2$ for each of the VFA and the ratio improved over the linear models, suggesting an improvement of prediction with a non linear approach. In cases such as isobutyrate, isovalerate, and butyrate the $R^2$ were greatly improved.

### Neural Net – Ruminal VFA MMoles/day

### Fit History

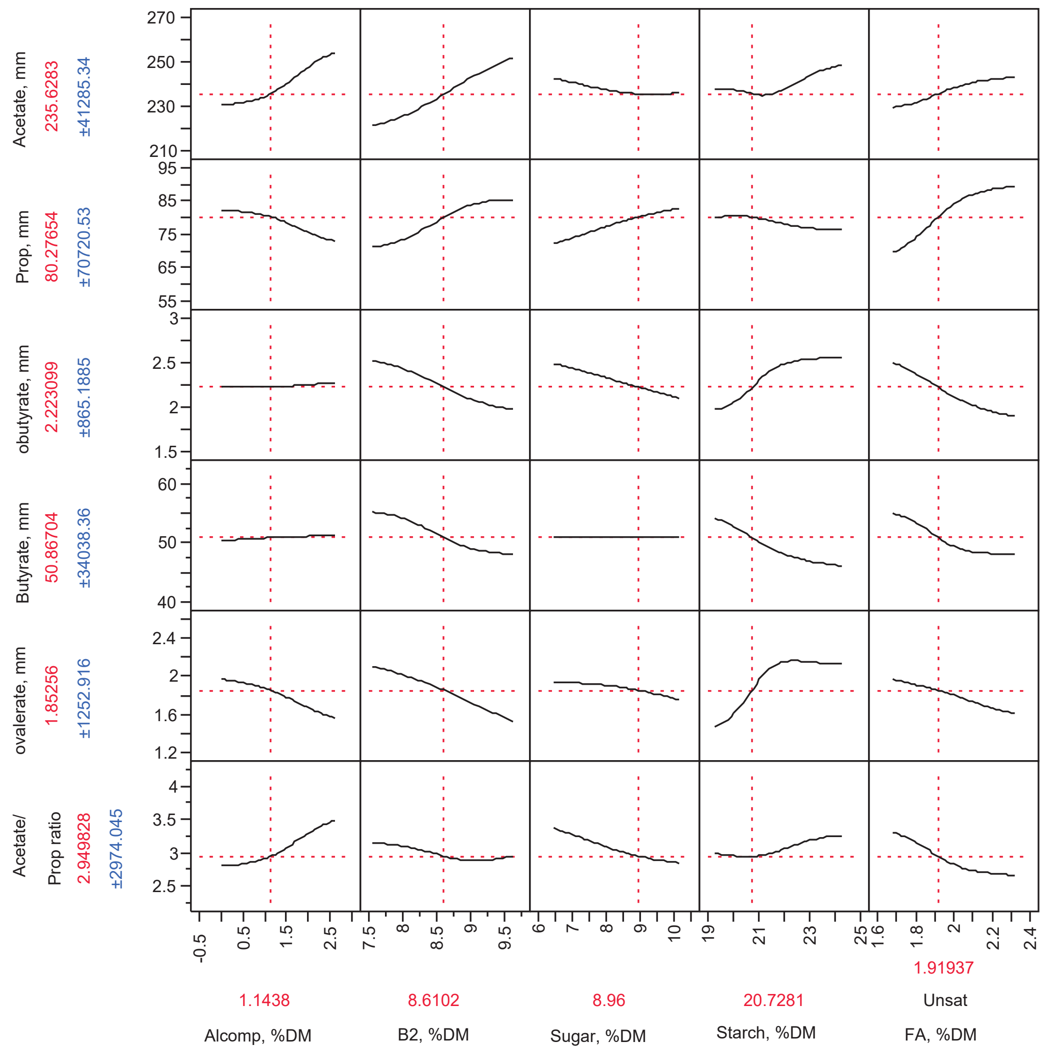
| Nodes | Penalty | RSquare |
|---|---|---|
| 3 | 0.01 | 0.66194 |

| Y | SSE | RMSE | SSE Scaled | RMSE Scaled | RSquare |
|---|---|---|---|---|---|
| Acetate, mm | 3634.7842 | 9.08893465 | 21.638182363 | 0.70126805 | 0.5396 |
| Prop, mm | 1293.175848 | 5.42128769 | 20.066918457 | 0.67532683 | 0.5730 |
| Isobutyrate, mm | 1.2398644223 | 0.16786523 | 12.52267128 | 0.53348493 | 0.7336 |
| Butyrate, mm | 331.39490079 | 2.7443947 | 12.433427745 | 0.53158057 | 0.7355 |
| Isovalerate, mm | 0.9391205951 | 0.14609466 | 8.8524731481 | 0.44854495 | 0.8116 |
| Acetate/Prop ratio | 1.6571225961 | 0.19406668 | 19.818147361 | 0.67112774 | 0.5783 |

The response curves below are similar to the ones above with some differences. An explanation was not given for the unsaturated fatty acids above. These are the sum of 18:1c, 18:2 and 18:3 expressed as a %DMI. These unsaturated fatty acids have been determined to have the most influence on ruminal fermentation and the generation of Trans fatty acids which have a negative impact on milk fat. It has been determined that when the unsats go above 2.5 to 2.6% DMI milk fat decreases. It seemed reasonable to include this variable in the model. It will be noted that the acetate/propionate ratio decreases as the unsats increase. As above Alcomp has a positive influence on acetate with a negative influence on propionate, resulting in an improved acetate:propionate ratio. This fits with the work done earlier with Alcomp and could be positive as part of the potential improvement in milk fat when Alcomp is fed.

The protein fraction B2 is a true protein fraction, which contributes significantly to the peptides produced in the rumen. The B2 in Alcomp is 5% of the product (see above) so will contribute little to the overall peptide pool. Peptides improve microbial efficiency as well as isoacids needed by the fiber bacteria for growth. Note that the B2 fraction has a negative impact on some of the other VFA in the fermentation which is in contrast to acetate and propionate.

**Prediction Profiler**




We can conclude that Alcomp is impacting ruminal fermentation and we need to be sensitive to the mixtures of the NFC CHO mixtures that we have in rations when Alcomp is included in the ration. We also, with further investigation might be able to develop tools to diagnose both the responses and non responses when Alcomp is used.

Presented below are the microbial and bypass protein responses. There was not much of an improvement in the non linear predictions over the linear. However, the overall $R^2$ were excellent for the 3 traits. The Alcomp responses were significant in all cases. It is interesting to note that as Alcomp increases in the ration the microbial yield increases and the bypass protein decreases. Overall, though, the total protein increases (NAN) which means that there will be a net increase of a higher quality protein to the small intestine because the microbial yield overcomes the decrease in the bypass protein. Again, the B2 protein fraction is important for the microbial yield. It seems that we do not want to go over 2.6% DMI in LCFA without a precipitous drop in microbial yield. Further, the starch needs to be lower in the ration with higher sugar and soluble fiber, controlling rumen pH and keeping the $NH_3$ above 5 mg% in these fermenters; the latter fitting with earlier work.

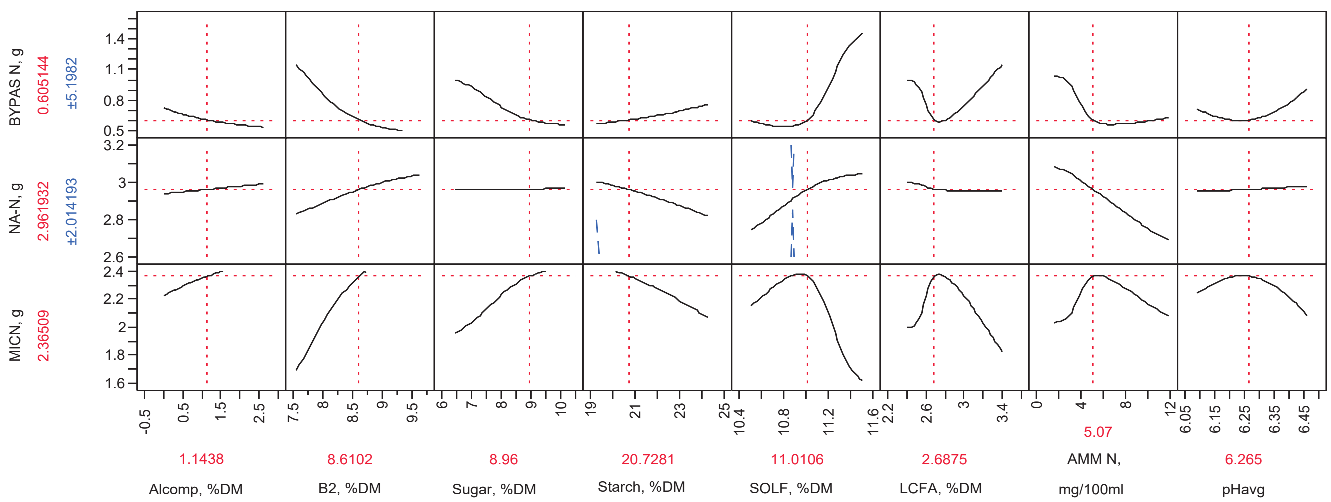
## Neural Net – Microbial and Bypass Protein

### Fit History

| Nodes | Penalty | RSquare |
|---|---|---|
| 3 | 0.01 | 0.88974 |

| Y | SSE | RMSE | SSE Scaled | RMSE Scaled | RSquare |
|---|---|---|---|---|---|
| BYPAS N, g | 0.3504048976 | 0.08923983 | 6.6159336864 | 0.38776556 | 0.8592 |
| NA-N, g | 0.0155634742 | 0.01880732 | 1.0322401818 | 0.15316659 | 0.9780 |
| MICN, g | 0.239112716 | 0.07371825 | 7.8980691587 | 0.42367626 | 0.8320 |

### Prediction Profiler




These data do fit some of the research done with cows. This would suggest that we need to push towards higher forage rations when feeding Alcomp at a little over 1% of the DM with recognition that we might need to reconsider the blend of protein and carbohydrate fractions that we should be formulating for.

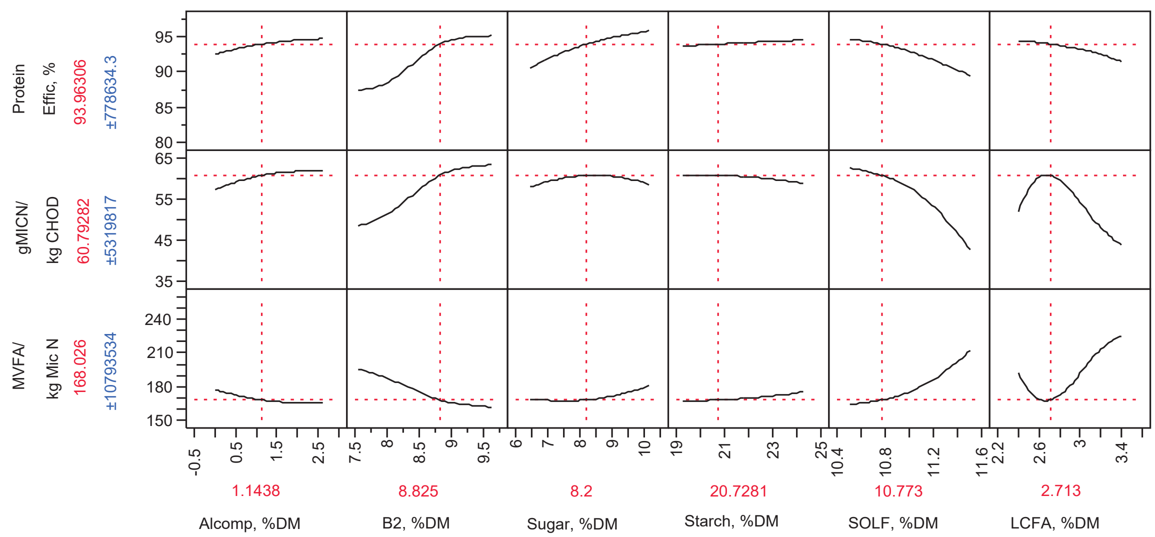
Below are the efficiencies that are usually examined. The $R^2$'s are all reasonable and in every case the inclusion of Alcomp significantly improved the prediction of the model. It is not unusual to have no improvement in efficiency. With Alcomp, there is an improvement.

## Neural Net – Protein and Microbial Efficiency

| Y | SSE | RMSE | SSE Scaled | RMSE Scaled | RSquare |
|---|---|---|---|---|---|
| Protein Effic, % | 100.85206272 | 1.51396576 | 12.634734114 | 0.53586663 | 0.7312 |
| gMICN/kg CHOD | 530.9478466 | 3.47375827 | 13.183777508 | 0.54738588 | 0.7195 |
| MVFA/kg Mic N | 7910.2518086 | 13.4081486 | 18.314138469 | 0.64515922 | 0.6103 |

Below are the profiler responses. In this profiler, the focus was on the moles of VFA produced/kg Mic N. This is an estimate of the degree of what we call fermentation coupling. In this case, the lower the number the higher the degree of coupling. Another way to look at this is that VFA are a waste product of fermentation by the bacteria. The vertical red dot lines were moved left or right to optimize the degree of coupling. Therefore, the less VFA produced by the bacteria the more that is going into microbial mass. The inclusion of Alcomp improved protein efficiency. It also improved g microbial N/kg of fermented CHO. It is interesting how sensitive the degree of coupling was to the LCFA inclusion in the ration. This might be an area for further examination. B2 continues to be important and this needs to be further examined. In this analysis it is suggested that there be total sugar of around 8%. This is higher than what we normally see in rations. We do have to remember, however, that the analyses coming from the WVA lab provides higher sugars than CVAS, due to the fructans which they include in the analyses. In CVAS terms this would probably translate into about 6% sugar. The residual sugars in a lot of rations with fermented forages is usually in the 3 to 4% range. This would mean adding 2 to 3% additional sugar, which at times can be difficult.

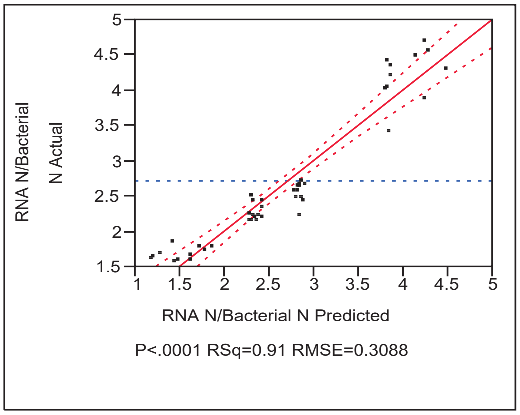
## Prediction Profiler




Starch is having little impact. This trait would probably be more sensitive if we had a measurement of its degradability. Soluble fiber is measured by indirect means as the difference after accounting for all of the other NFC fractions. In CNCPS 6.1, this number can potentially now mean more as the plant organic acids such as malic acid are now accounted for. The soluble fiber is mostly pectins and beta glucans. If using CVAS analysis for sugar then the fructans are also in the soluble fiber fraction. Formulating for 10.5 %SOLF is a high number in our typical Midwest and Northeast rations. More typical in Western rations where more alfalfa is used.

The question is if there is so much activity with the addition of Alcomp, given that there are changes in microbial yield and efficiency as well as improved fermentation coupling and improvements in fiber digestibility, is the population changing? Let's understand that there is a small protozoal population in the fermenters. There is, however, a large fungal population. This fungal population is sensitive to sugars. Below is a crude estimate of changes in the bacterial ecology. This assay does not take into account the small protozoal population or the fungal population. The $R^2$ for the model was 0.91. Alcomp was marginally significant, ranking next to the bottom for the Sorted Parameter estimates. It is expected that there could be some non linear components but with this high $R^2$, the change will be modest.

### Response RNA N/Bacterial N
### Actual by Predicted Plot




### Summary of Fit

| | |
|---|---|
| RSquare | 0.91125 |
| RSquare Adj | 0.900427 |
| Root Mean Square Error | 0.308848 |
| Mean of Response | 2.709582 |
| Observations (or Sum Wgts) | 47 |

### Analysis of Variance

| Source | DF | Sum of Squares | Mean Square | F Ratio |
|---|---|---|---|---|
| Model | 5 | 40.155234 | 8.03105 | 84.1944 |
| Error | 41 | 3.910863 | 0.09539 | **Prob > F** |
| C. Total | 46 | 44.066098 | | <.0001* |

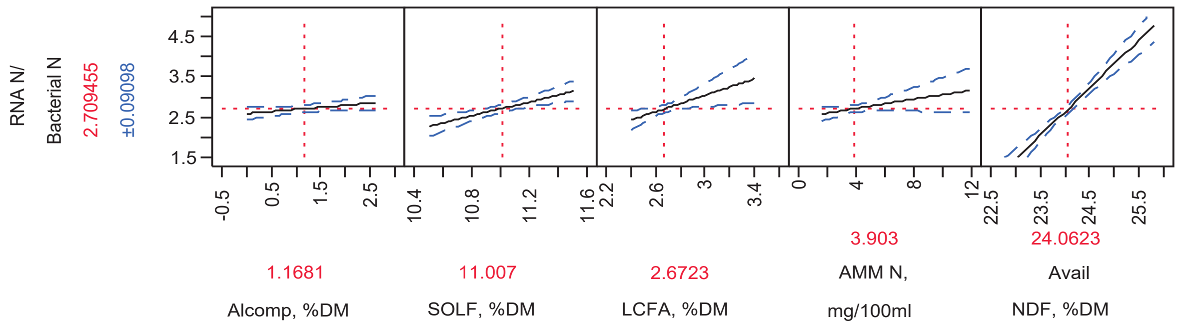
### Parameter Estimates

| Term | Estimate | Std Error | t Ratio | Prob>\|t\| |
|---|---|---|---|---|
| Intercept | -38.73812 | 5.863936 | -6.61 | <.0001* |
| Alcomp, %DM | 0.1035031 | 0.055812 | 1.85 | 0.0709 |
| SOLF, %DM | 0.884398 | 0.23101 | 3.83 | 0.0004* |
| LCFA, %DM | 1.0205653 | 0.405915 | 2.51 | 0.0159* |
| AMM N, mg/100ml | 0.0575015 | 0.034159 | 1.68 | 0.0999 |
| Avail NDF, %DM | 1.1902609 | 0.118428 | 10.05 | <.0001* |

### Sorted Parameter Estimates

| Term | Estimate | Std Error | t Ratio | t Ratio | Prob>\|t\| |
|---|---|---|---|---|---|
| Avail NDF, %DM | 1.1902609 | 0.118428 | 10.05 | | <.0001* |
| SOLF, %DM | 0.884398 | 0.23101 | 3.83 | | 0.0004* |
| LCFA, %DM | 1.0205653 | 0.405915 | 2.51 | | 0.0159* |
| Alcomp, %DM | 0.1035031 | 0.055812 | 1.85 | | 0.0709 |
| AMM N, mg/100ml | 0.0575015 | 0.034159 | 1.68 | | 0.0999 |

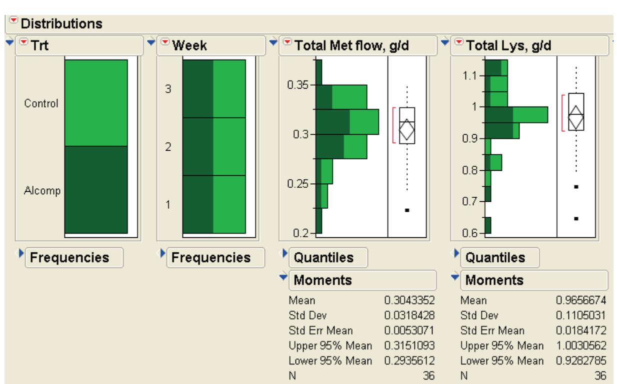
## Prediction Profiler



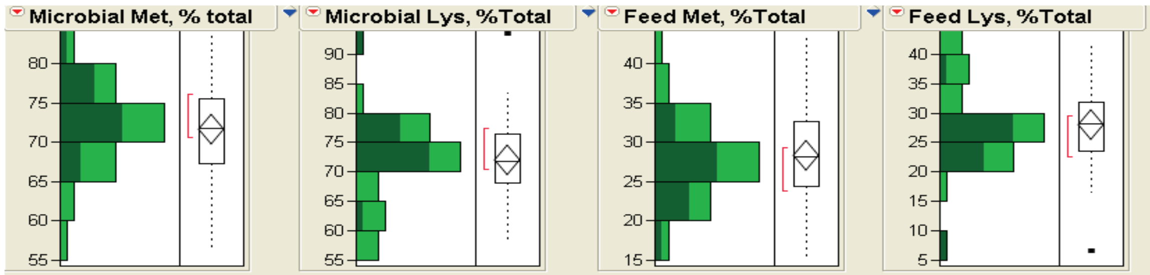

The profiler above shows a strong effect from the available fiber. This probably reflects the increase, in the combined studies, of the increase in the forage in the rations and is correlated with the impact of the positive effect from Alcomp relative to the acetate/propionate ratio. It should be noted that for LCFA and AMM N that the dashed blue confidence lines spread out as their concentrations increase. This could mean that a non linear representation of these variables could be more appropriate.

In the second study, there was an addendum to examine the impact of Alcomp on the changes in the amino acid flows out of the fermenters. Total amino acid flow, microbial amino acid flow and bypass feed amino acid flow was measured. As one would expect with some of the changes shown above, that there would be differences in the AA flowing out of the fermenters. The fermentation lab provided an extensive report on all of the amino acids, which we should refer to for more information. This was a 3 week study with the %forage in the ration being increased each week. The microbial population had one week at each forage level to adapt before samples were taken.

We will focus on Methionine (Met) and Lysine (Lys) because of the current focus in the industry on these AA due to the recent availability of rumen protected Lys (RPLys) in addition to the rumen protected Met (RPMet).




The highlighted (dark green) represents Alcomp for Lys & Met over all 3 weeks.




Quantiles

Moments

| | Microbial Met, % total | Microbial Lys, %Total | Feed Met, %Total | Feed Lys, %Total |
|---|---|---|---|---|
| Mean | 71.708269 | 72.016156 | 28.291731 | 27.983844 |
| Std Dev | 5.5316182 | 7.4872335 | 5.5316182 | 7.4872335 |
| Std Err Mean | 0.9219364 | 1.2478722 | 0.9219364 | 1.2478722 |
| Upper 95% Mean | 73.579899 | 74.549472 | 30.163361 | 30.517159 |
| Lower 95% Mean | 69.836639 | 69.482841 | 26.420101 | 25.450528 |
| N | 36 | 36 | 36 | 36 |

Above are the Met and Lys for microbial and feed as a % of the total Met and Lys flow out of the fermenters with the Alcomp treatment highlight regardless of week. Both microbial Met & Lys as a % of total tend to be higher because of Alcomp inclusion, with the feed escape being less. However, the total Met and Lys flow is higher in g/d because of Alcomp inclusion.

Below are the statistics related to Lysine and Methionine flow out of the fermenters. Again, for more detail on the other amino acids, refer to the detailed report from the West Virginia Fermentation Lab.

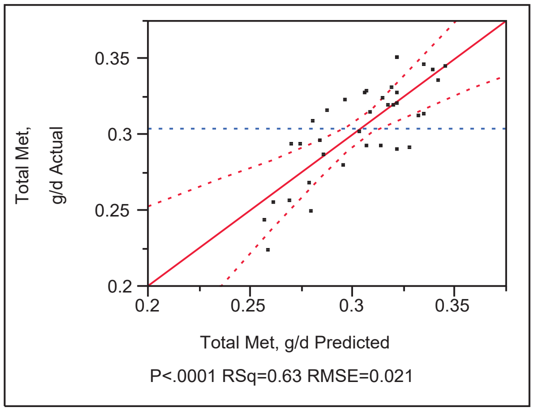
The approach taken was to place the treatment and weeks into a categorical rather than a continuous criteria. Ammonia N and pH were added to the model to look at the modifying effects of these two variables, because part of the question being asked is about the microbial area.

Total Met and Lys flow is the first question then Met and Lys, as a % of total flow first for microbial and then as the contribution from the feed. The latter approach is a different approach with the use of fermenter data and may be innovative and more precise than the measurements that we make in vivo with cows, given the added assumptions that we need to make relative to flow and to endogenous contributions.

Total flow reflects the combination of the microbial and protein escaping fermentation. It was noted earlier in this report that there was increased protein degraded in the rumen with the addition of Alcomp, and there was an increase in microbial flow and microbial efficiency. The proteins degraded will be those more sensitive to being degraded like the proteins in soybean meal which have large B2 pools with degradation rates close to the rates of passage. There are many instances where the total protein flowing to the small intestine will be less than before the addition of the ruminal additive. Most frequently it will be the same but the quality is assumed to improve with the greater proportion of the protein escaping being microbial protein.

Below Alcomp did not impact total flow of either Met or Lys on average. However, with the increase in forage in the ration from week one to week two there was a significant increase in flow of both Met and Lys. It can be seen in both of the means tables that Alcomp had little impact but there was an increase in the second week for both Met and Lys with little change in the 3$^{rd}$ week. The ammonia level had no effect and the rumen pH had a significant effect on the outcome.

## Least Squares Fit
## Response Total Met, g/d
## Actual by Predicted Plot




## Summary of Fit

| | |
|---|---|
| RSquare | 0.628833 |
| RSquare Adj | 0.566972 |
| Root Mean Square Error | 0.020954 |
| Mean of Response | 0.304335 |
| Observations (or Sum Wgts) | 36 |

## Analysis of Variance

| Source | DF | Sum of Squares | Mean Square | F Ratio |
|---|---|---|---|---|
| Model | 5 | 0.02231653 | 0.004463 | 10.1652 |
| Error | 30 | 0.01317228 | 0.000439 | **Prob > F** |
| C. Total | 35 | 0.03548882 | | <.0001* |

## Parameter Estimates

| Term | Estimate | Std Error | t Ratio | Prob>\|t\| |
|---|---|---|---|---|
| Intercept | 1.2948297 | 0.364851 | 3.55 | 0.0013* |
| Alcomp, %DM[2.21-0] | -0.003459 | 0.008529 | -0.41 | 0.6880 |
| Week[2-1] | 0.0516785 | 0.008667 | 5.96 | <.0001* |
| Week[3-2] | -0.005663 | 0.01021 | -0.55 | 0.5833 |
| AMM N, mg/100ml | 0.0026375 | 0.002196 | 1.20 | 0.2392 |
| pHavg | -0.162889 | 0.057995 | -2.81 | 0.0087* |

## Effect Tests

| Source | Nparm | DF | Sum of Squares | F Ratio | Prob > F |
|---|---|---|---|---|---|
| Alcomp, %DM | 1 | 1 | 0.00007220 | 0.1644 | 0.6880 |
| Week | 2 | 2 | 0.01699760 | 19.3561 | <.0001* |
| AMM N, mg/100ml | 1 | 1 | 0.00063310 | 1.4419 | 0.2392 |
| pHavg | 1 | 1 | 0.00346374 | 7.8887 | 0.0087* |

## Effect Details
## Alcomp, %DM
## Least Squares Means Table

| Level | Least Sq Mean | Std Error | Mean |
|---|---|---|---|
| 0 | 0.27349981 | 0.00849505 | 0.305641 |
| 2.21 | 0.27004105 | 0.00703157 | 0.303029 |

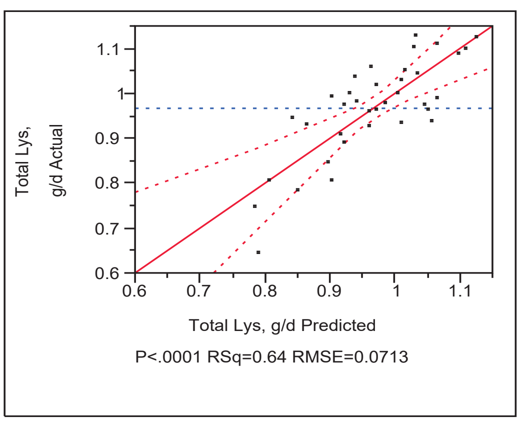
## Week
## Least Squares Means Table

| Level | Least Sq Mean | Std Error | Mean |
|---|---|---|---|
| 1 | 0.27349981 | 0.00849505 | 0.273798 |
| 2 | 0.32517833 | 0.00791425 | 0.327280 |
| 3 | 0.31951572 | 0.00735578 | 0.311928 |

## Scaled Estimates
Continuous factors centered by mean, scaled by range/2

| Term | Scaled Estimate | Plot Estimate | Std Error | t Ratio | Prob>\|t\| |
|---|---|---|---|---|---|
| Intercept | 0.2734998 | | 0.008495 | 32.20 | <.0001* |
| Alcomp, %DM[2.21-0] | -0.003459 | | 0.008529 | -0.41 | 0.6880 |
| Week[2-1] | 0.0516785 | | 0.008667 | 5.96 | <.0001* |
| Week[3-2] | -0.005663 | | 0.01021 | -0.55 | 0.5833 |
| AMM N, mg/100ml | 0.0134816 | | 0.011227 | 1.20 | 0.2392 |
| pHavg | -0.020361 | | 0.007249 | -2.81 | 0.0087* |

## Response Total Lys, g/d
## Actual by Predicted Plot




## Summary of Fit

| | |
|---|---|
| RSquare | 0.643208 |
| RSquare Adj | 0.583743 |
| Root Mean Square Error | 0.071294 |
| Mean of Response | 0.965667 |
| Observations (or Sum Wgts) | 36 |

## Analysis of Variance

| Source | DF | Sum of Squares | Mean Square | F Ratio |
|---|---|---|---|---|
| Model | 5 | 0.27489576 | 0.054979 | 10.8165 |
| Error | 30 | 0.15248667 | 0.005083 | **Prob > F** |
| C. Total | 35 | 0.42738243 | | <.0001* |

## Parameter Estimates

| Term | Estimate | Std Error | t Ratio | Prob>\|t\| |
|---|---|---|---|---|
| Intercept | 5.6286034 | 1.241368 | 4.53 | <.0001* |
| Alcomp, %DM[2.21-0] | -0.02831 | 0.02902 | -0.98 | 0.3371 |
| Week[2-1] | 0.1599065 | 0.029488 | 5.42 | <.0001* |
| Week[3-2] | 0.002219 | 0.03474 | 0.06 | 0.9495 |
| AMM N, mg/100ml | 0.0117426 | 0.007473 | 1.57 | 0.1266 |
| pHavg | -0.758217 | 0.197322 | -3.84 | 0.0006* |

## Effect Tests

| Source | Nparm | DF | Sum of Squares | F Ratio | Prob > F |
|---|---|---|---|---|---|
| Alcomp, %DM | 1 | 1 | 0.00483720 | 0.9517 | 0.3371 |
| Week | 2 | 2 | 0.17469091 | 17.1842 | <.0001* |
| AMM N, mg/100ml | 1 | 1 | 0.01254918 | 2.4689 | 0.1266 |
| pHavg | 1 | 1 | 0.07504959 | 14.7651 | 0.0006* |

## Effect Details

### Alcomp, %DM

### Least Squares Means Table

| Level | Least Sq Mean | Std Error | Mean |
|---|---|---|---|
| 0 | 0.87247837 | 0.02890356 | 0.978415 |
| 2.21 | 0.84416832 | 0.02392421 | 0.952920 |

### Week

### Least Squares Means Table

| Level | Least Sq Mean | Std Error | Mean |
|---|---|---|---|
| 1 | 0.8724784 | 0.02890356 | 0.86823 |
| 2 | 1.0323849 | 0.02692744 | 1.03620 |
| 3 | 1.0346040 | 0.02502729 | 0.99258 |

## Scaled Estimates

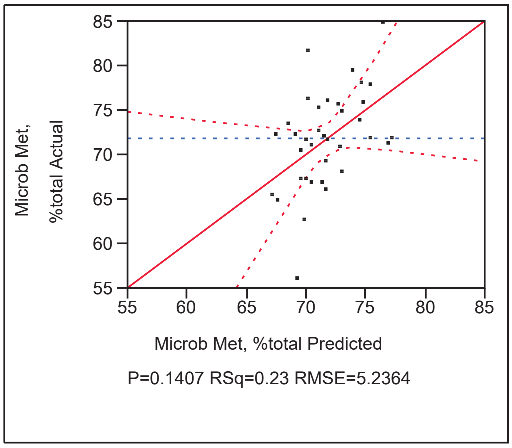
Continuous factors centered by mean, scaled by range/2

| Term | Scaled Estimate | Plot Estimate | Std Error | t Ratio | Prob>\|t\| |
|---|---|---|---|---|---|
| Intercept | 0.8724784 | | 0.028904 | 30.19 | <.0001* |
| Alcomp, %DM[2.21-0] | -0.02831 | | 0.02902 | -0.98 | 0.3371 |
| Week[2-1] | 0.1599065 | | 0.029488 | 5.42 | <.0001* |
| Week[3-2] | 0.002219 | | 0.03474 | 0.06 | 0.9495 |
| AMM N, mg/100ml | 0.0600221 | | 0.0382 | 1.57 | 0.1266 |
| pHavg | -0.094777 | | 0.024665 | -3.84 | 0.0006* |

Both Met and Lys as a % of the total flow of Met and Lys are shown below. The model for Microbial Met was not significant but Alcomp in the model significantly improved the % of the total flow of Met as microbial Met. This is very apparent from the Scaled Estimates.

The Microb Lys, %total model was significant. Alcomp had a highly significant and positive effect on the Lys content of the total digesta flowing out of the fermenters. There were differences among weeks as well with the 2nd week being lower.

## Response Microb Met, %total
## Actual by Predicted Plot




## Summary of Fit

| | |
|---|---|
| RSquare | 0.231919 |
| RSquare Adj | 0.103906 |
| Root Mean Square Error | 5.236354 |
| Mean of Response | 71.70827 |
| Observations (or Sum Wgts) | 36 |

## Analysis of Variance

| Source | DF | Sum of Squares | Mean Square | F Ratio |
|---|---|---|---|---|
| Model | 5 | 248.3760 | 49.6752 | 1.8117 |
| Error | 30 | 822.5821 | 27.4194 | **Prob > F** |
| C. Total | 35 | 1070.9580 | | 0.1407 |

## Parameter Estimates

| Term | Estimate | Std Error | t Ratio | Prob>\|t\| |
|---|---|---|---|---|
| Intercept | -24.83084 | 91.17469 | -0.27 | 0.7872 |
| Alcomp, %DM[2.21-0] | 4.9584675 | 2.131438 | 2.33 | 0.0269* |
| Week[2-1] | -1.133978 | 2.165809 | -0.52 | 0.6044 |
| Week[3-2] | -0.775598 | 2.55154 | -0.30 | 0.7632 |
| AMM N, mg/100ml | -0.839797 | 0.548888 | -1.53 | 0.1365 |
| pHavg | 15.520066 | 14.49267 | 1.07 | 0.2928 |

## Effect Tests

| Source | Nparm | DF | Sum of Squares | F Ratio | Prob > F |
|---|---|---|---|---|---|
| Alcomp, %DM | 1 | 1 | 148.39092 | 5.4119 | 0.0269* |
| Week | 2 | 2 | 15.38295 | 0.2805 | 0.7574 |
| AMM N, mg/100ml | 1 | 1 | 64.18579 | 2.3409 | 0.1365 |
| pHavg | 1 | 1 | 31.44476 | 1.1468 | 0.2928 |

## Effect Details
## Alcomp, %DM
## Least Squares Means Table

| Level | Least Sq Mean | Std Error | Mean |
|---|---|---|---|
| 0 | 70.243553 | 2.1228779 | 69.8888 |
| 2.21 | 75.202021 | 1.7571599 | 73.5278 |

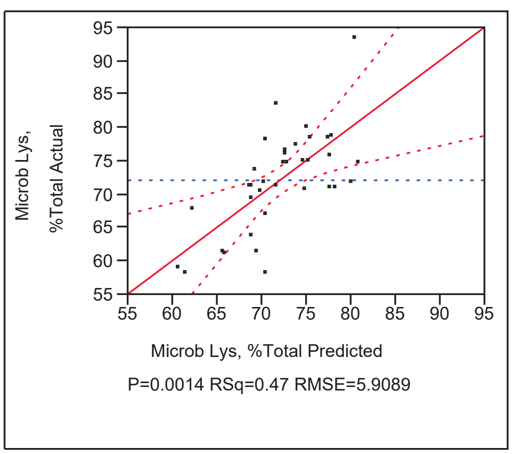
## Week
## Least Squares Means Table

| Level | Least Sq Mean | Std Error | Mean |
|---|---|---|---|
| 1 | 70.243553 | 2.1228779 | 73.0465 |
| 2 | 69.109575 | 1.9777376 | 71.3683 |
| 3 | 68.333977 | 1.8381779 | 70.7100 |

## Scaled Estimates
Continuous factors centered by mean, scaled by range/2

| Term | Scaled Estimate | Plot Estimate | Std Error | t Ratio | Prob>\|t\| |
|---|---|---|---|---|---|
| Intercept | 70.243553 | | 2.122878 | 33.09 | <.0001* |
| Alcomp, %DM[2.21-0] | 4.9584675 | | 2.131438 | 2.33 | 0.0269* |
| Week[2-1] | -1.133978 | | 2.165809 | -0.52 | 0.6044 |
| Week[3-2] | -0.775598 | | 2.55154 | -0.30 | 0.7632 |
| AMM N, mg/100ml | -4.292624 | | 2.805643 | -1.53 | 0.1365 |
| pHavg | 1.9400083 | | 1.811584 | 1.07 | 0.2928 |

## Response Microb Lys, %Total
## Actual by Predicted Plot



## Parameter Estimates

| Term | Estimate | Std Error | t Ratio | Prob>\|t\| |
|---|---|---|---|---|
| Intercept | -133.8233 | 102.8852 | -1.30 | 0.2033 |
| Alcomp, %DM[2.21-0] | 6.7255314 | 2.405202 | 2.80 | 0.0089* |
| Week[2-1] | -6.580134 | 2.443987 | -2.69 | 0.0115* |
| Week[3-2] | 5.2430409 | 2.879262 | 1.82 | 0.0786 |
| AMM N, mg/100ml | -0.754299 | 0.619388 | -1.22 | 0.2328 |
| pHavg | 32.84825 | 16.35412 | 2.01 | 0.0537 |

## Summary of Fit

| | |
|---|---|
| RSquare | 0.466142 |
| RSquare Adj | 0.377165 |
| Root Mean Square Error | 5.908916 |
| Mean of Response | 72.01616 |
| Observations (or Sum Wgts) | 36 |

## Analysis of Variance

| Source | DF | Sum of Squares | Mean Square | F Ratio |
|---|---|---|---|---|
| Model | 5 | 914.5947 | 182.919 | 5.2389 |
| Error | 30 | 1047.4586 | 34.915 | **Prob > F** |
| C. Total | 35 | 1962.0533 | | 0.0014* |


## Parameter Estimates

| Term | Estimate | Std Error | t Ratio | Prob>\|t\| |
|---|---|---|---|---|
| Intercept | -133.8233 | 102.8852 | -1.30 | 0.2033 |
| Alcomp, %DM[2.21-0] | 6.7255314 | 2.405202 | 2.80 | 0.0089* |
| Week[2-1] | -6.580134 | 2.443987 | -2.69 | 0.0115* |
| Week[3-2] | 5.2430409 | 2.879262 | 1.82 | 0.0786 |
| AMM N, mg/100ml | -0.754299 | 0.619388 | -1.22 | 0.2328 |
| pHavg | 32.84825 | 16.35412 | 2.01 | 0.0537 |


## Effect Tests

| Source | Nparm | DF | Sum of Squares | F Ratio | Prob > F |
|---|---|---|---|---|---|
| Alcomp, %DM | 1 | 1 | 273.00185 | 7.8190 | 0.0089* |
| Week | 2 | 2 | 280.63608 | 4.0188 | 0.0284* |
| AMM N, mg/100ml | 1 | 1 | 51.78182 | 1.4831 | 0.2328 |
| pHavg | 1 | 1 | 140.85931 | 4.0343 | 0.0537 |

## Effect Details

### Alcomp, %DM

### Least Squares Means Table

| Level | Least Sq Mean | Std Error | Mean |
|---|---|---|---|
| 0 | 71.292466 | 2.3955423 | 68.9728 |
| 2.21 | 78.017998 | 1.9828511 | 75.0595 |

### Week

### Least Squares Means Table

| Level | Least Sq Mean | Std Error | Mean |
|---|---|---|---|
| 1 | 71.292466 | 2.3955423 | 74.4418 |
| 2 | 64.712332 | 2.2317601 | 67.3571 |
| 3 | 69.955373 | 2.0742751 | 74.2496 |

## Effect Tests

| Source | Nparm | DF | Sum of Squares | F Ratio | Prob > F |
|---|---|---|---|---|---|
| Alcomp, %DM | 1 | 1 | 273.00185 | 7.8190 | 0.0089* |
| Week | 2 | 2 | 280.63608 | 4.0188 | 0.0284* |
| AMM N, mg/100ml | 1 | 1 | 51.78182 | 1.4831 | 0.2328 |
| pHavg | 1 | 1 | 140.85931 | 4.0343 | 0.0537 |

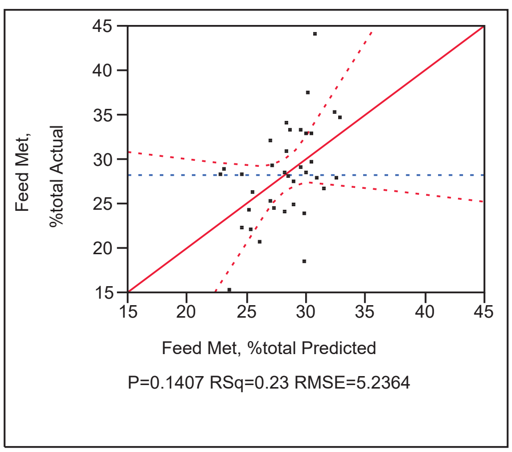
## Scaled Estimates

Continuous factors centered by mean, scaled by range/2

| Term | Scaled Estimate | Plot Estimate | Std Error | t Ratio | Prob>\|t\| |
|---|---|---|---|---|---|
| Intercept | 71.292466 | | 2.395542 | 29.76 | <.0001* |
| Alcomp, %DM[2.21-0] | 6.7255314 | | 2.405202 | 2.80 | 0.0089* |
| Week[2-1] | -6.580134 | | 2.443987 | -2.69 | 0.0115* |
| Week[3-2] | 5.2430409 | | 2.879262 | 1.82 | 0.0786 |
| AMM N, mg/100ml | -3.855601 | | 3.166003 | -1.22 | 0.2328 |
| pHavg | 4.1060312 | | 2.044265 | 2.01 | 0.0537 |

Below are the Feed AA flow. If, in fact, as shown above, the feed protein increases in ruminal degradability the amino acid profile of the protein escaping will be different from the amino acid profile of the protein consumed. This is shown in the detailed West Virginia report. Alcomp had a significant negative effect on the feed Met and Lys as a % of the total, because it positively influenced the microbial yield. However, Feed Lys, which was a significant model, as a % of total Lys increased in week 2 and then decreased in week 3.

## Response Feed Met, %total
## Actual by Predicted Plot



## Summary of Fit

| | |
|---|---|
| RSquare | 0.231919 |
| RSquare Adj | 0.103906 |
| Root Mean Square Error | 5.236354 |
| Mean of Response | 28.29173 |
| Observations (or Sum Wgts) | 36 |

## Analysis of Variance

| Source | DF | Sum of Squares | Mean Square | F Ratio |
|---|---|---|---|---|
| Model | 5 | 248.3760 | 49.6752 | 1.8117 |
| Error | 30 | 822.5821 | 27.4194 | **Prob > F** |
| C. Total | 35 | 1070.9580 | | 0.1407 |

## Parameter Estimates

| Term | Estimate | Std Error | t Ratio | Prob>\|t\| |
|---|---|---|---|---|
| Intercept | 124.83084 | 91.17469 | 1.37 | 0.1811 |
| Alcomp, %DM[2.21-0] | -4.958468 | 2.131438 | -2.33 | 0.0269* |
| Week[2-1] | 1.1339784 | 2.165809 | 0.52 | 0.6044 |
| Week[3-2] | 0.7755982 | 2.55154 | 0.30 | 0.7632 |
| AMM N, mg/100ml | 0.8397974 | 0.548888 | 1.53 | 0.1365 |
| pHavg | -15.52007 | 14.49267 | -1.07 | 0.2928 |

## Effect Tests

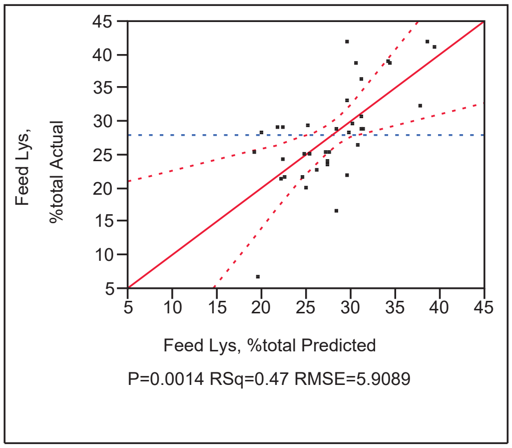
| Source | Nparm | DF | Sum of Squares | F Ratio | Prob > F |
|---|---|---|---|---|---|
| Alcomp, %DM | 1 | 1 | 148.39092 | 5.4119 | 0.0269* |
| Week | 2 | 2 | 15.38295 | 0.2805 | 0.7574 |
| AMM N, mg/100ml | 1 | 1 | 64.18579 | 2.3409 | 0.1365 |
| pHavg | 1 | 1 | 31.44476 | 1.1468 | 0.2928 |

## Scaled Estimates

Continuous factors centered by mean, scaled by range/2

| Term | Scaled Estimate | Plot Estimate | Std Error | t Ratio | Prob>\|t\| |
|---|---|---|---|---|---|
| Intercept | 29.756447 | | 2.122878 | 14.02 | <.0001* |
| Alcomp, %DM[2.21-0] | -4.958468 | | 2.131438 | -2.33 | 0.0269* |
| Week[2-1] | 1.1339784 | | 2.165809 | 0.52 | 0.6044 |
| Week[3-2] | 0.7755982 | | 2.55154 | 0.30 | 0.7632 |
| AMM N, mg/100ml | 4.2926242 | | 2.805643 | 1.53 | 0.1365 |
| pHavg | -1.940008 | | 1.811584 | -1.07 | 0.2928 |

## Response Feed Lys, %total
## Actual by Predicted Plot




## Summary of Fit

| | |
|---|---|
| RSquare | 0.466142 |
| RSquare Adj | 0.377165 |
| Root Mean Square Error | 5.908916 |
| Mean of Response | 27.98384 |
| Observations (or Sum Wgts) | 36 |

## Analysis of Variance

| Source | DF | Sum of Squares | Mean Square | F Ratio |
|---|---|---|---|---|
| Model | 5 | 914.5947 | 182.919 | 5.2389 |
| Error | 30 | 1047.4586 | 34.915 | **Prob > F** |
| C. Total | 35 | 1962.0533 | | 0.0014* |

## Parameter Estimates

| Term | Estimate | Std Error | t Ratio | Prob>\|t\| |
|---|---|---|---|---|
| Intercept | 233.82333 | 102.8852 | 2.27 | 0.0304* |
| Alcomp, %DM[2.21-0] | -6.725531 | 2.405202 | -2.80 | 0.0089* |
| Week[2-1] | 6.5801338 | 2.443987 | 2.69 | 0.0115* |
| Week[3-2] | -5.243041 | 2.879262 | -1.82 | 0.0786 |
| AMM N, mg/100ml | 0.7542994 | 0.619388 | 1.22 | 0.2328 |
| pHavg | -32.84825 | 16.35412 | -2.01 | 0.0537 |

## Effect Tests

| Source | Nparm | DF | Sum of Squares | F Ratio | Prob > F |
|---|---|---|---|---|---|
| Alcomp, %DM | 1 | 1 | 273.00185 | 7.8190 | 0.0089* |
| Week | 2 | 2 | 280.63608 | 4.0188 | 0.0284* |
| AMM N, mg/100ml | 1 | 1 | 51.78182 | 1.4831 | 0.2328 |
| pHavg | 1 | 1 | 140.85931 | 4.0343 | 0.0537 |

## Effect Details

### Alcomp, %DM

### Least Squares Means Table

| Level | Least Sq Mean | Std Error | Mean |
|---|---|---|---|
| 0 | 28.707534 | 2.3955423 | 31.0272 |
| 2.21 | 21.982002 | 1.9828511 | 24.9405 |

### Week

### Least Squares Means Table

| Level | Least Sq Mean | Std Error | Mean |
|---|---|---|---|
| 1 | 28.707534 | 2.3955423 | 25.5582 |
| 2 | 35.287668 | 2.2317601 | 32.6429 |
| 3 | 30.044627 | 2.0742751 | 25.7504 |

### Scaled Estimates

Continuous factors centered by mean, scaled by range/2

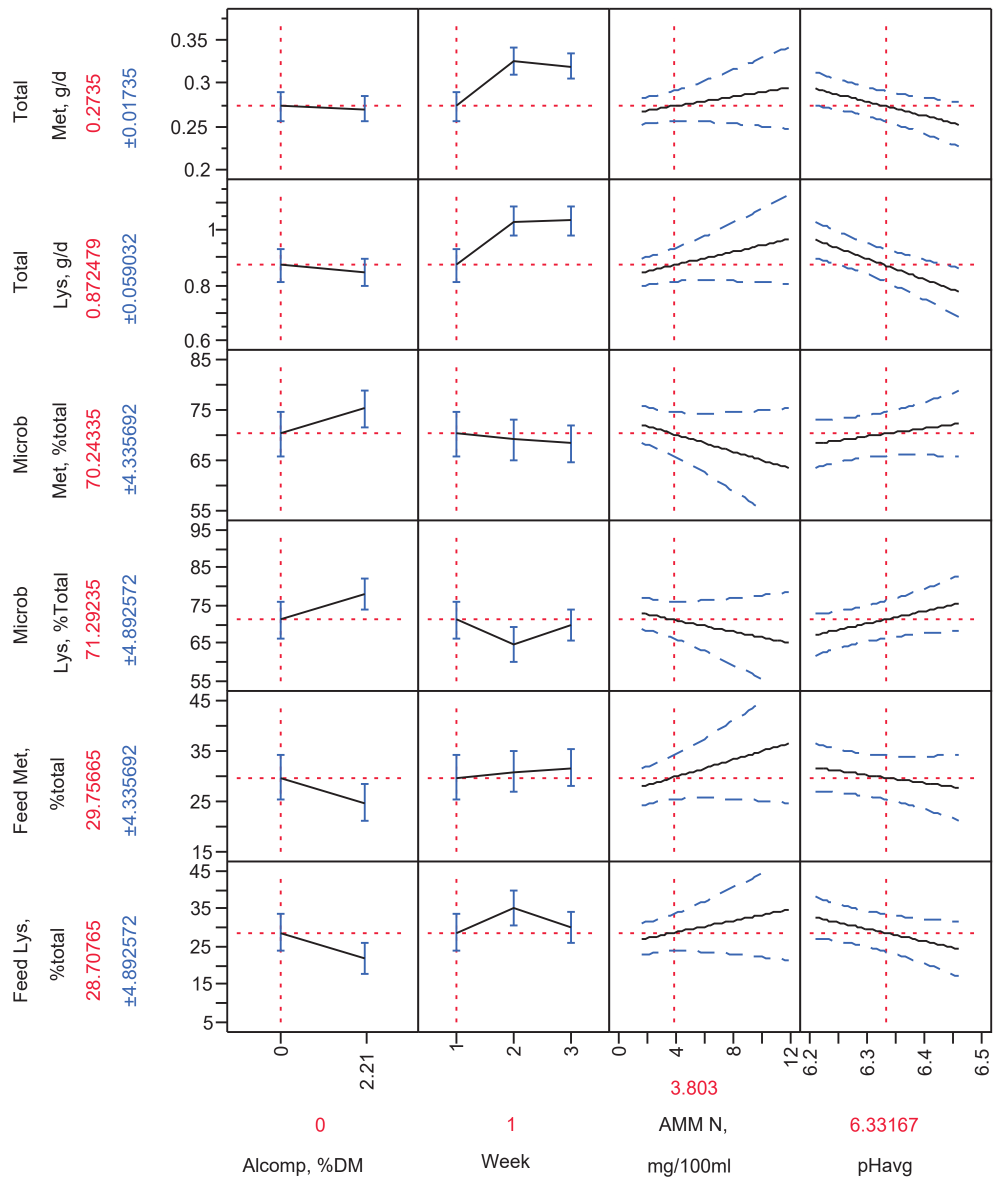
| Term | Scaled Estimate | Plot Estimate | Std Error | t Ratio | Prob>\|t\| |
|---|---|---|---|---|---|
| Intercept | 28.707534 | | 2.395542 | 11.98 | <.0001* |
| Alcomp, %DM[2.21-0] | -6.725531 | | 2.405202 | -2.80 | 0.0089* |
| Week[2-1] | 6.5801338 | | 2.443987 | 2.69 | 0.0115* |
| Week[3-2] | -5.243041 | | 2.879262 | -1.82 | 0.0786 |
| AMM N, mg/100ml | 3.8556012 | | 3.166003 | 1.22 | 0.2328 |
| pHavg | -4.106031 | | 2.044265 | -2.01 | 0.0537 |

Below are the prediction profilers which provide a visual presentation of the changes that occurred in the AA study. Alcomp had an impact on total Met and Lys flow, mainly through the increase in the microbial yield and through a higher proportion of the protein (NAN) being from the microbial yield. This is the first time that this type of measurement has been made. This approach shows some significant opportunities, especially if we can analyze the amino acids of the feedstuffs being provided and then assess the feed and microbial AA flowing from the fermenters. This might provide us a model that will allow us to translate to our nutrition models and improve our prediction of AA flow to the small intestine.

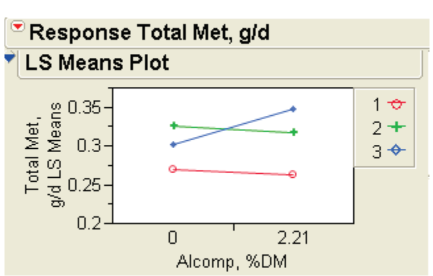
The rumen ammonia and the rumen pH had variable effects in the models. Given the variance shown in the plot, one would assume that there were some non linearity in response. Indeed, when this was examined, it was found to be the case. However, it was decided to use the linear models for this presentation because the overall $R^2$ were not greatly improved. B2, starch and sugar were also examined. These variables did not improve the models. This is of interest. This is probably because of the narrow range the rations in these experiments. Available fiber was not used because it is correlated with week. The week by treatment interaction was not tested, even though it can be seen below that there were some interactions. Again, this was looked at in the in depth report.

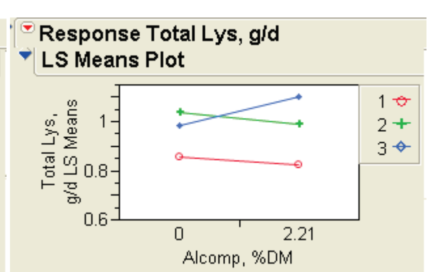
I think the important two points that can be taken away from this limited AA report is that with the increase in forage (weeks), the Met and Lys improved. This was enhanced by the inclusion of Alcomp. Apparently, the dietary Lys degraded in the rumen in these studies was more extensive than the Met.

## Prediction Profiler

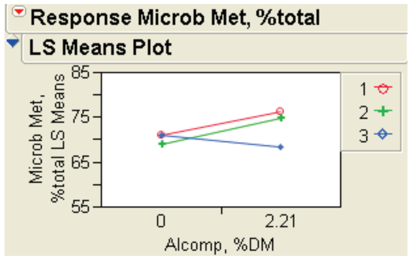


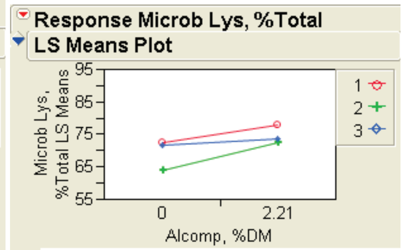

There were some questions about the third week in the second experiment, so it was decided to break out the treatment by week interaction. One can see that week one was clearly lower in both Met and Lys output. One can see that there was a clear added response to Alcomp in week two and a slight decline or no change in week 3 in total Met and Lys output.



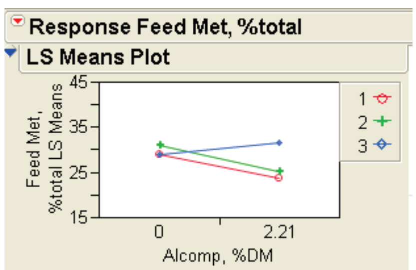



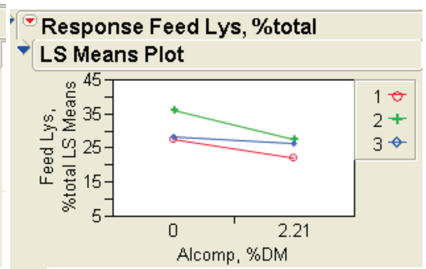
In terms of the microbial Met as a % of the total Met flow, Week 1 & 2 increased with Alcomp and week 3 decreased. Lys was different. All increased with Alcomp but the response in week 3 was weak, with week two showing the greatest response.




Feed Met Dropped in Weeks 1 and 2, reflecting the enhancement in the microbial yield for weeks 1 and 2. Week 3 showed an increase. Feed Lys decreased for all 3 weeks but week 3 decreased the least.




One could conclude from the breakout of the week by treatment interactions that there was something different in week 3 which needs to be further examined.

**Summary**

Alcomp does need some refinement in what is used for analyses of the product. The anomaly of the two analyses of the distillers needs to be resolved. The procedure to resolve this can be discussed.

Alcomp as modeled does affect the fermentation in the rumen. VFA patterns were modified, fiber digestion enhanced, microbial yield and microbial efficiency was enhanced. The changes in the microbial ecology are suggested. The question could be raised that our assumptions on a constant AA profile from microbial contributions. The AA study done with the second study was exciting and will allow us to further drill into how Alcomp is influencing animal performance.

It is suggested that there needs to be discussion on establishing nutritional parameters with the use of Alcomp. It is also suggested that more work needs to be done to establish the nutritional parameters for different inclusion levels of Alcomp as well as the maximum that should be used under different dietary restrictions. It is suggested that we need to examine closely the above models as well as the examination of the suggestions made by Steve Mehen, based on the combined studies, as well as the work in Kansas and in Minnesota, to design a controlled field study with a high producing herd or herds.