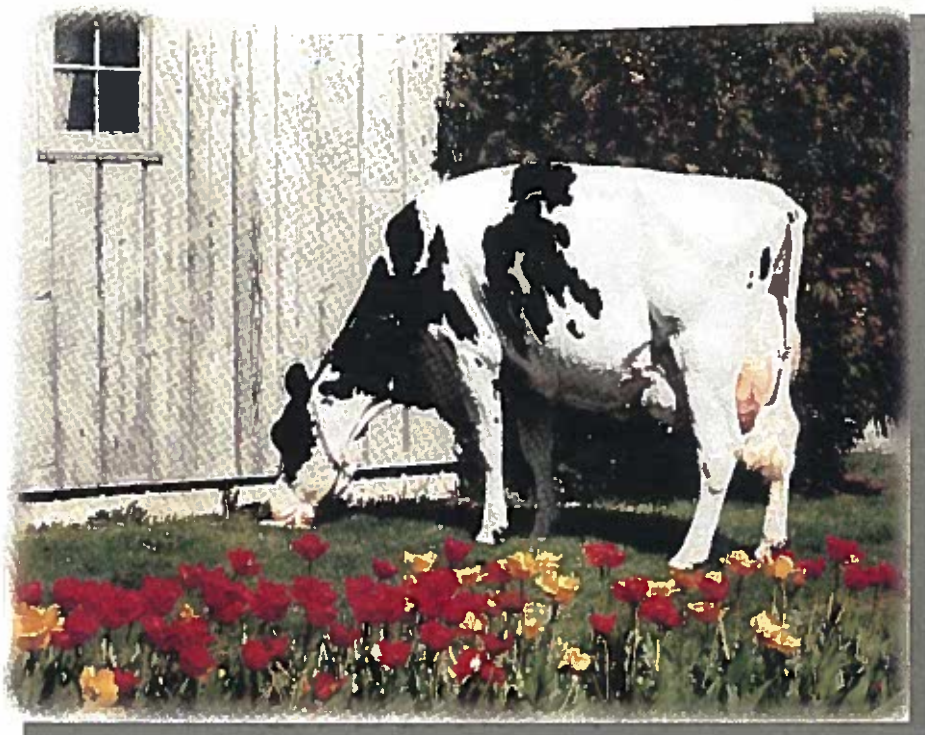


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*Abbreviations that may be found in this publication include:*

AA = amino acids	NE <sub>L</sub> = net energy for lactation
ADF = acid detergent fiber	NDF = neutral detergent fiber
BCS = body condition score	NFC = nonfiber carbohydrates
BW = body weight	NRC = National Research Council
CP = crude protein	NSC = nonstructural carbohydrates
CV = coefficient of variation	OM = organic matter
DE = digestible energy	r = correlation coefficient
DIM = days in milk	R <sup>2</sup> = coefficient of determination
DHI = Dairy Herd Improvement	RDP = rumen degradable protein
DM = dry matter	RFV = relative feed value
DMI = dry matter intake	RMSE = root mean square error
ECM = energy corrected milk	RUP = rumen undegradable protein
FA = fatty acids	SCC = somatic cell count
FCM = fat corrected milk	SD = standard deviation
ME = metabolizable energy	SE = standard error
MCP = microbial crude protein	SEM = standard error of mean
MP = metabolizable protein	TDN = total digestible nutrients
NEFA = non esterified fatty acids	TMR = total mixed ration
NE <sub>g</sub> = net energy for gain	VFA = volatile fatty acids

Note: Most of the units of measure in this publication are expressed in US equivalents; however, in some cases, metric units are used. Use the following to make conversions:

$$1.0 \text{ lb} = 0.454 \text{ kg} = 454 \text{ g}$$

$$1.0 \text{ ft} = 0.3 \text{ m} = 30 \text{ cm}$$

$$^{\circ}\text{F} = (^{\circ}\text{C} \times 1.8) + 32$$

*Abbreviations for metric units are:*

ppm = parts per million

mg = milligrams

g = grams

kg = kilograms

cm = centimeters

mm = millimeters

m = meters

km = kilometers

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## Minimizing Subclinical Metabolic Diseases

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### Abstract

Subclinical ketosis is a common disease in lactating dairy cows with a lactational incidence rate above 40% in many herds. This condition is associated with increased clinical disease risk, reduced milk production, and impaired reproductive performance. On a herd basis, subclinical ketosis is much more costly than clinical ketosis. Prevention is achieved largely through effective dry cow programs that encompass both good nutrition and excellent cow management. However, certain additives are helpful in reducing subclinical ketosis. These include propylene glycol, rumen protected choline, and where approved for dairy cattle – ionophores. Effective monitoring programs are a critical component to managing subclinical ketosis. Monitoring is useful for assessment of the transition cow program and for identification of individual animals to treat.

### Introduction

Most periparturient abnormalities have some metabolic element as a component of the sufficient cause of clinical disease. The metabolic disturbance of milk fever can be measured through low serum calcium concentrations. Negative energy balance, fat mobilization, and subsequent elevations in ketone body concentrations play a contributing role in the expression of fatty liver syndrome, clinical ketosis, and abomasal displacement. A

negative energy balance may also increase the risk of retained placenta, metritis, and mastitis through impaired immune function. A third category of metabolic disease in early lactation might include rumen acidosis, which is marked by low rumen pH. Thus, calcium homeostasis, energy balance, and rumen pH are important considerations for disease prevention in transition dairy cows (Goff and Horst, 1997).

In general, subclinical disease incidence is far more common than clinical disease, frequently going unnoticed and may be associated with significant clinical disease risks, impaired production, and reduced reproductive performance. Of the three major subclinical metabolic diseases, the most information exists for subclinical ketosis. It is associated with both losses in milk production and increased risk of periparturient disease. Prevention depends on several factors, including proper transition cow nutrition, management of body condition, and may be helped through the use of certain feed additives, such as niacin, propylene glycol, rumen protected choline, and ionophores. It is commonly accepted that subclinical hypocalcemia is an important disease, but very little information is published on the impact of this problem on subsequent risk of disease or production loss. Subclinical rumen acidosis is also thought to be a major problem on many dairy farms, but it is difficult to measure and very little controlled research exists for this syndrome. This article will focus on the

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importance, prevention aspects, and monitoring strategies for subclinical ketosis.

### Is subclinical ketosis a disease?

Elevated levels of circulating ketone bodies occur in early lactation in response to the homeorhetic drive to sustain high levels of milk production, at a time when dry matter intake is reduced (Baird, 1982). The three major ketone bodies are acetone, acetoacetate, and beta-hydroxybutyrate (**BHBA**). Subclinical ketosis is simply a condition marked by increased levels of circulating ketone bodies without the presence of the clinical signs of ketosis. Subclinical ketosis has been associated with increased risk of specific periparturient diseases (ketosis, displaced abomasum, metritis, and mastitis), decreased milk production, and impaired reproductive performance. If these impacts are true, then prevention or reduction in incidence should ameliorate some or all of the negative effects of this condition. Administration of a monensin controlled release capsule three weeks precalving has decreased the incidence of subclinical ketosis, clinical ketosis, and displaced abomasum, and improved milk production (particularly in cows and herds at increased risk of subclinical ketosis).

#### *Association with periparturient disease*

Cows in early lactation with subclinical ketosis had an increased risk of metritis four days later (Dohoo and Martin, 1984). However, most studies have identified ketosis to be a result rather than a cause of metritis. Cows having subclinical ketosis are at increased risk of subsequently developing clinical ketosis (Dohoo and Martin, 1984). The relationship between displaced abomasum and ketosis has been identified as bi-directional (Curtis et al., 1985; Grohn et al., 1989). That is, ketosis may be a cause of displacement, and abomasal displacement may lead to ketosis. Correa et al. (1993) found that ketosis increased the risk of abomasal displacement, but not the reverse.

However, ketosis, as an inciting or predisposing cause of abomasal displacement, can be further supported by some recent Guelph research. Elevated BHBA concentrations above 1000 mmol/L increased the likelihood of abomasal displacement (Geishauser et al., 1997). Cows with concentrations of BHBA at or above 1400  $\mu\text{mol/L}$  in the first two weeks post calving were three times more likely to subsequently develop either clinical ketosis or abomasal displacement (Duffield, 1997).

Two studies have found a relationship between the diagnosis of ketosis prior to identifying mastitis (Dohoo and Martin, 1984; Syvajarvi et al., 1986). Mastitis increased the risk of ketosis in Finnish Ayrshires (Grohn et al., 1989). Hyperketonemic cows with BHBA blood levels above 1400  $\mu\text{mol/L}$  were found to suffer a more severe experimental mastitis than normal cows (Kremer et al., 1993). There may be some important immune function implications associated with decreased energy balance and subclinical ketosis. Recently, two separate studies have identified subclinical ketosis as a risk factor for the subsequent occurrence of clinical mastitis (Leslie et al., 2000).

#### *Impact on milk production*

In general, there is consensus that a negative association between hyperketonemia and milk production exists. In one study, the loss of production associated with a positive milk ketone test was 1.0 to 1.4 kg/day of milk for a lactation (Dohoo and Martin, 1984). Test day milk production was negatively correlated with milk acetone levels in several Scandinavian projects (Andersson and Emanuelson, 1985; Gustafsson et al., 1993; Steen et al., 1996). Kauppinen (1984) reported that subclinically ketotic cows had significantly higher annual milk yields than nonketotic cows. Herdt et al. (1981) found higher levels of BHBA in higher producing cows, but individual milk tests were not collected on the same day but preceded blood

measurement for BHBA. It is possible that higher milk yields put cows at increased risk of developing subclinical ketosis. Increased levels of milk production may be associated with increased fat mobilization and a greater risk of hyperketonemia.

#### *Effect on milk components*

Milk fat and milk protein are significantly altered in hyperketonemia. Milk fat percentage was increased in subclinically ketotic cows (Miettinen, 1994; Miettinen and Setälä, 1993). The association between milk fat and hyperketonemia is, presumably, because of increased availability of BHBA and fatty acids for milk fat synthesis. It is unclear whether increased levels of circulating ketones cause increased milk fat, or if cows that are prone to higher milk fat yields are more susceptible to subclinical ketosis. Milk protein percentage has been reported to be lower in cows with subclinical ketosis (Miettinen, 1994; Miettinen and Setälä, 1993). This may be the result of a reduced energy supply, since milk protein percentage is positively associated with net energy balance. Recently, we have identified an impact of subclinical ketosis on milk fat percentage at first DHI test postcalving only, but a reduction in milk protein percentage for the first three DHI tests.

#### *Impact on reproductive performance*

Increasing the degree of negative energy balance in early lactation has been shown to increase the interval from calving to first ovulation (Butler and Smith, 1989). Butler and Smith (1989) suggested that cows with a longer interval from calving to first ovulation experience a decrease in pregnancy at first service because conception risk is related to the number of ovulatory cycles that occur prior to insemination (Stevenson and Call, 1983; Whitmore et al., 1974). Since hyperketonemia is a symptom of a disturbed energy metabolism, many authors have investigated the relationship

between subclinical ketosis and reproductive performance. No effect of either subclinical or clinical ketosis on individual cow fertility was found in two studies (Andersson and Emanuelson, 1985; Kaupinnen, 1984). However, significant correlations between the herd prevalence of hyperketonemia and herd mean intervals from both calving to first service and calving to last service have been noted (Andersson and Emanuelson, 1985). A link between subclinical ketosis and the increased incidence of cystic ovaries has also been reported (Andersson and Emanuelson, 1985; Dohoo and Martin, 1984). Miettinen and Setälä (1993) found an increased interval from calving to conception in cows with high milk and fat yields. The associations between fertility and increased fat and milk yields do not necessarily imply a relationship between impaired fertility and hyperketonemia. The duration of either clinical or subclinical ketosis may be too short to exert a negative effect on calving interval. However, Whitaker et al. (1993) found cows with a better energy status at 14 days postpartum had a reduced interval from calving to the onset of cyclicity and fewer services per conception. No effect was observed when energy status was evaluated at 21 days postpartum or at first service. This study was only conducted on 24 cows within one herd. In a much larger dataset, Cook et al. (2001) reported significantly longer calving to conception intervals and higher culling rates in cows that had high milk acetone concentrations in early lactation. Initial screening of our 1010 cow dataset from 1995 indicates a significant reduction in 1<sup>st</sup> service conception rate for cows identified to be subclinically ketotic in the second week postpartum.

#### *Cost of subclinical ketosis*

When negative impacts of milk production losses, increased risk of disease, and reduced reproductive performance are considered, the cost of one cow with subclinical ketosis is estimated to be \$78 U.S. (Geishauer

et al., 2001). This number will vary depending on several variables, including the value assigned for milk, impaired reproduction, and metabolic disease. Regardless, the individual disease value for subclinical ketosis is less than clinical disease. However, because the subclinical form is more prevalent, the cost at the herd level is much higher. For example, if an average dairy herd has an incidence rate for clinical ketosis of 5% and the disease costs \$145.00, a 100-cow dairy herd would have a cost of clinical ketosis of \$725 in a year. Whereas, an average 100-cow dairy would have a subclinical ketosis incidence of 41%, with an annual cost of \$3198.

### How common is subclinical ketosis?

Before a program is instituted, the veterinarian and farm manager need to know what the average incidence of subclinical ketosis is for the herd so that a reasonable and achievable target can be set. In a recent trial conducted at Guelph, the median incidence of subclinical ketosis (BHBA > 1400  $\mu\text{mol/L}$ ) in untreated cows was 41% for the first nine weeks of lactation (Duffield et al., 1998). This was roughly equivalent to two cows identified as subclinically ketotic per 10 cows examined in each of the first and the second weeks post-calving. The range across 25 herds for the total nine weeks was 8 to 80%. The four highest herds had incidence rates above 65% and also had the largest milk production response to prophylactic treatment.

### Prevention

#### *General Guidelines*

Since ketosis occurs in early lactation, recommendations for prevention have focused on the nutritional management of the dry and transition cow. Detailed recommendations for nutrition during the dry period can be found elsewhere (Oetzel, 1998). It is a common recommendation to divide the dry period into

two feeding groups: far-off and close-up (Radostits et al, 1994). Typically, far-off diets follow NRC (2001) guidelines for dry cows. The close-up diet is usually balanced according to recommendations that are halfway between those for the dry cow and those for the early lactation cow and should be fed starting at least three weeks before expected calving (Oetzel, 1998). The goals of the transition diet (specifically designed to prevent subclinical ketosis) are to maximize dry matter intake (**DMI**) to provide adequate energy density (Oetzel, 1998). Avoidance of ketogenic feedstuffs (Tveit et al., 1992) and increased frequency of feeding concentrates (Andersson, 1988; Gustafsson et al., 1993) have been advocated as preventive measures against subclinical ketosis. The reduction of overconditioning cows in late lactation and the early dry period, as well as lead feeding with concentrates about three weeks prior to calving, have also been suggested as aids in prophylaxis (Andersson, 1988; Lean et al, 1991). Maximizing DMI and maintenance of a consistent intake through the last three weeks prior to calving is likely the hallmark of a successful transition cow program. Recent work at Guelph (Tera Osborne, MS candidate, personal communication) indicates that a DMI of less than 12 kg/day per cow in the last three weeks prior to calving substantially increases the subsequent risk of subclinical ketosis (Odds Ratio 5.7,  $P < 0.05$ ). Achieving group DMI targets above an average of 12 kg/day per cow are possible, and based on the above finding, should be a goal for the close-up group. More important than ration formulation and ration ingredients, close attention should be paid to cow comfort and environmental issues. These factors include, but are not limited to, adequate pen space or stall space per cow, adequate feed bunk space, sufficient and comfortable bedding, adequate water supply, and minimization of heat stress.

### *Feed Additives*

In addition to good nutrition, certain feed additives have been found beneficial in reducing subclinical ketosis, when administered prophylactically. Niacin fed prior to calving at the rate of 3 to 6 g/day may be helpful in reducing blood levels of BHBA (Dufva et al., 1983; Fronk and Schultz, 1979). Propylene glycol has been used successfully for the prevention of subclinical ketosis (Emery et al., 1964; Sauer et al., 1973). Treatment of cows for eight weeks starting at calving with either 3 or 6% propylene glycol in a concentrate mixture significantly reduced the incidence of positive milk ketone tests (Fisher et al., 1973). Precalving oral treatment with 300 g/day of propylene glycol for 10 days lowered serum non-esterified fatty acids (NEFA) concentrations and improved some measures of reproductive performance in one study (Formigoni et al., 1996). A dose of propylene glycol of 1 L/day as an oral drench for nine days prior to calving decreased BHBA, and NEFA and increased glucose concentrations (Struder et al., 1993). It appears that a bolus of propylene glycol is necessary for maximum effect, since mixing in a total mixed ration is not as efficacious as either an oral drench or when mixed with a small quantity of grain (Christenson et al., 1995). Schultz (1958) reported that sodium propionate could be given to prevent clinical ketosis in dairy cattle. Propylene glycol requires repeated daily oral administration and sodium propionate may reduce feed intake (Sauer et al., 1989). Ionophores have been proposed as potential prophylactic agents for reducing hyperketonemia (Lean et al., 1991; Tyler et al., 1992). In contrast to propylene glycol and sodium propionate, ionophores are relatively inexpensive and much easier to administer. Rumen protected choline reduced liver triglycerides and increased liver glycogen (Piepenbrink and Overton, 2000). More recently, preliminary Guelph research shows reductions in NEFA and BHBA concentrations postcalving in cows receiving

rumen protected choline (Reashure™; Balchem Encapsulates, Slate Hill, NY) during transition compared to control cows. The cost benefit of this prevention tool needs to be investigated.

### *Ionophores*

The gluconeogenic potential of monensin has attracted researchers to investigate its possible role as an antiketogenic agent in dairy cattle. Rogers and Hope-Cawdery (1980) first described the beneficial effects of monensin for reducing the incidence of ketosis in a herd with a clinical ketosis problem. The antiketogenic properties of monensin were later investigated in a Canadian trial involving two levels of monensin and three groups of 12 Holstein cows (Sauer et al., 1989). Monensin included at 30 g/ton of total ration (high group), decreased the incidence of subclinical ketosis and significantly reduced blood BHBA concentrations in the first three weeks postpartum (Sauer et al., 1989). The incidence of subclinical ketosis, defined as total blood ketones > 9 mg/100 ml (900 μmol/L), was reduced by 50% and blood BHBA concentrations were reduced by 40% for the high monensin group. Based on the average feed intakes observed in this trial, the low monensin group received approximately 208 mg/day of monensin and the high group 399 mg/day. The monensin treatment commencing at two to four weeks prior to calving reduced serum BHBA and NEFA in lactating dairy cows during the first 28 days postpartum when monensin was fed at 300 or 450 mg/day, but not by a daily dose of 150 mg/day of monensin (Thomas et al., 1993). Serum glucose was not influenced by monensin feeding. Australian cows treated with a monensin controlled release capsule (CRC) during the first week postcalving had significantly lower plasma BHBA concentrations and tended to have higher glucose concentrations than controls (Abe et al., 1994). A CRC that delivers 335 mg/day of monensin sodium for 95 days reduced the incidence of subclinical ketosis by 50% and also decreased

the duration of the condition when it was administered 3 weeks prior to expected calving (Duffield et al., 1998).

The most closely linked diseases occurring subsequent to subclinical ketosis are displaced abomasum and clinical ketosis. Estimates of milk production loss range from 300 to 450 kg (660 to 990 lb) for a lactation (Dohoo and Martin, 1984; Gustafsson et al., 1993). These losses must be weighed against the cost of any prophylactic measure. Administration of a monensin CRC precalving reduced the incidence of clinical ketosis by 50%, abomasal displacement by 40%, and multiple illness by 40% (more than one disease) (Duffield et al., 1999b). The milk production response depended on body condition and was 0.85 kg/day (1.9 lb/day) at peak lactation in cows with a precalving body condition score (**BCS**) of 3.25 to 3.75 and was 1.2 kg/day (2.6 lb/day) for the first 90 days of lactation in fat cows (**BCS**<sup>3</sup> 4.0)(Duffield et al., 1999a). No milk production response was noted in thin cows, presumably because they had the lowest BHBA concentrations and were at decreased risk of subclinical ketosis. A subsequent Canadian study conducted in 45 dairy herds confirmed that monensin CRC reduces the incidence of displaced abomasum (Duffield et al., 2002). A pooled summary of the two Canadian projects showed that monensin CRC reduced the incidence of displaced abomasum and clinical ketosis by 40% each. In addition to the impact of monensin on displaced abomasum and clinical ketosis, pooled analysis of the two Canadian CRC studies showed that the incidence of retained placenta tended to be lowered by 25% in monensin treated cows ( $P = 0.09$ ). Monensin is currently not approved in the United States for use in lactating dairy cows.

## Monitoring subclinical ketosis

*When do I test cows?*

By most definitions, the theoretical testing period for transition cows would extend from three weeks prior to calving until three weeks after calving. Practically however, the most important time periods are: during the last week prior to calving and within the first two weeks after calving.

*Precalving*

It is unusual for cows to develop subclinical ketosis precalving because the etiology of the condition depends on the homeorhetic drive for milk production. However, cows in an energy deficit precalving will start mobilizing energy reserves in the final week before parturition. This can be measured via serum or plasma NEFA. The challenge for this precalving sample is predicting when the animal is going to calve. In most cases, a serum bank needs to be established and then samples are submitted retrospectively once the actual calving date is known.

*Postcalving*

A ketone testing program should commence after calving. The primary risk period for subclinical ketosis is the first month of calving. Our work at Guelph has indicated that the first two weeks postcalving is the time of peak incidence. In addition, the median days to diagnosis of clinical ketosis and displaced abomasum were 11 days. Thus, in order to try to prevent subclinical disease from becoming clinical disease (if that is possible), cows must be identified early. For these reasons, a subclinical ketosis monitoring program should focus on the first two to three weeks of lactation.

## What test do I use?

### *NEFA*

This test should only be used precalving on samples obtained within one week of parturition. Unfortunately, these restrictions make the utility of this test limited. However, it may serve useful in certain situations, such as a herd investigation or an intervention follow-up. The data for this variable is frequently right skewed and thus averages can be very misleading. One suggested threshold is 0.5 units/L. In recent work, cows within one week of calving with serum NEFA above this threshold were at a 3.5 times greater risk of subsequently developing a displaced abomasum. Whole herd interpretation is best made by calculating a proportion of cows above a threshold value; however, at this point, there are not a lot of good data on an appropriate goal for this parameter. In a multi-herd 1060 cow study near Guelph, 30% of cows were above 0.5 U/L during the last week prior to calving.

### *Serum BHBA*

In contrast to NEFA, serum BHBA should only be used postcalving. The first two weeks are the primary risk period for subclinical ketosis, defined by a serum concentration of 1400  $\mu\text{mol/L}$  BHBA or greater. Although BHBA is the most stable of the ketones, it is the most subject to variation associated with feed intake, thus all samples on a given farm should always be taken at the same time of day. In addition, hemolysis is known to artificially elevate values; therefore, hemolyzed samples should be avoided. Other disadvantages of serum BHBA is the cost (approximately \$5.00 per sample) and the laboratory turn around time (minimum 24 hours). However, all things considered, serum BHBA analysis is the gold standard from which to compare cowside tests. A reasonable goal is to have less than two cows per 10 with BHBA above 1400  $\mu\text{mol/L}$  in the first two weeks post-calving.

### *Milk Ketone Tests*

Most milk ketone tests measure acetone and acetoacetate through a chemical reaction with nitroprusside which causes a color change from white to either pink or purple. These tests in general are poorly sensitive in milk (< 40%) but highly specific (> 90%). One exception is the milk ketone test that measures BHBA. It is marketed in Europe as “Ketolac BHBA”, in Japan as “Sanketopaper”, and in Canada as “Keto-Test”. This test has a much higher sensitivity in milk (> 60%) and reasonably good specificity (> 70%, up to 90%). This is a semi-quantitative test that allows choosing a lower threshold for screening to increase sensitivity and a higher threshold for diagnosis to increase specificity.

### *Urine Ketone Tests*

The urine ketone tests are based on the same nitroprusside reaction as the milk powder ketone tests. These tests are highly sensitive (approaching 100%) but are poorly specific. Thus, they are great tests for ruling out subclinical ketosis with a negative test result. However, their use overestimates a subclinical ketosis problem because of a high probability of false positive reactions. If the urine test was used to evaluate the goal of less than two cows per 10 with BHBA above 1400  $\mu\text{mol/L}$  in the first two weeks post-calving, an adjustment of the goal to less than 5 cows per 10 with positive urine ketone tests would be required (Table 1). More work needs to be done to fully assess the utility of urine ketone tests.

## **Selection and Interpretation of Cowside Tests**

It is most likely that in screening a group of fresh cows, there would be two possible actions resulting from a test. One action might be to treat positive animals with the goal to prevent subsequent development of clinical disease. In this case, a high predictive value of a positive test is desired so that normal animals

are not unnecessarily treated. The second action might be to compare the percentage of positive reactors to a goal for determining the effectiveness of either the transition ration or some prophylactic measure in reducing the incidence of subclinical ketosis. In this situation, the apparent prevalence is the parameter that actually would be used. Note from Table 1 that the urine ketone test would substantially overestimate the prevalence of subclinical ketosis, while the Ketocheck™ test would grossly underestimate the prevalence. This does not preclude these tests from being used. However, the impact of the inherent sensitivity and specificity of the test must be remembered when establishing goals and intervention thresholds.

### Herd Disease Records

Herd records (computerized or paper) are important tools for monitoring the incidence of periparturient disease. Producers should set goals for minimizing the incidence of metabolic disease. Herd consultants should periodically review herd performance relative to the goals. In addition, intervention levels should also be considered. Several diseases are associated with increasing age, and this must be taken into account when assessing herd performance. For example, in monitoring and comparing herd incidence of milk fever and clinical ketosis, it is important to stratify this by parity. A high proportion of first lactation animals will give a herd a much lower incidence of milk fever and clinical ketosis, since risk increases with age.

Can herd incidence of certain diseases be used to decide whether a herd has a problem with subclinical ketosis? Herd level analysis of our 1995/1996 dataset involving 25 dairy herds indicates that the herd incidence of displaced abomasum is positively associated with the probability of a herd having a high incidence (> 20% in the first two weeks of lactation) of subclinical ketosis. The only other predictive variable was precalving body condition score

(higher average increased risk). If greater than 10% of the herd had a BCS > 4.0 at three weeks precalving, that herd was extremely likely to have a problem with subclinical ketosis.

### DHI Test Day Data

Since milk fat and milk protein percentages are altered in subclinical ketosis, these parameters have been investigated for their utility in defining subclinical ketosis. Among all protein and fat parameters, a protein to fat ratio of < 0.75 was the best test for diagnosing subclinical ketosis, at the cow level, in a Canadian study (Duffield et al., 1997). However, the protein to fat ratio was not a good test overall, having a sensitivity of 58% and a specificity of 69%.

Using data from a 25 herd study conducted in Guelph in 1995, the median cumulative herd incidence of subclinical ketosis was 41% in the first two months postcalving. Summary data for each herd from each cow's first DHI test postcalving were used to assess the protein to fat ratio as a test at the herd level for classifying a herd as a high or low incidence herd for subclinical ketosis. A herd mean protein to fat ratio of < 0.78 yielded a sensitivity of 69% and a specificity of 75% for identifying herds with subclinical ketosis problems. Further, if more than 40% of cows in the herd at 1<sup>st</sup> DHI test had a protein to fat ratio of less than or equal to 0.75, those herds were likely to be problem herds. This test had a sensitivity of 69% and a specificity of 83%. Although more work needs to be done on herd level indicators of subclinical ketosis, herd level protein to fat ratios appear to be better indicators of herd level issues than individual cow protein to fat ratios are of identifying cows with subclinical ketosis problems.

A recent study was conducted through the Ontario DHI testing facility to evaluate the utility of milk acetone measurements for diagnosing subclinical ketosis. At the cow level,

test day milk acetone values were not found to be useful in identifying cows at risk for developing clinical metabolic disease (displaced abomasum or ketosis) or clinical lameness. This is not an issue with the acetone test methodology but more likely a problem with the timing of the milk sample collection relative to the occurrence of the disease event. The highest prevalence of subclinical ketosis occurs within two weeks of calving. Since most of our DHI testing programs have an interval of 30 to 45 days and cows less than five days in milk on test day are not sampled, the probability of testing all cows within two weeks of calving is low. Therefore, from an implementation standpoint, the testing of routine DHI samples for the purpose of identifying cows at risk of subclinical or clinical disease is inefficient.

### Conclusions

Subclinical ketosis is an important and common disease in lactating dairy cows. Prevention depends largely on effective dry cow nutrition and management. However, certain feed additives, such as ionophores and rumen protected choline, may be beneficial. Given the cost of subclinical ketosis, the fact it is a common problem in early lactation, and the strong association with clinical disease, monitoring programs for subclinical ketosis during the first few weeks of lactation may be warranted. There are several cow-side tests for subclinical ketosis available; however, all of the current tests have their strengths and weaknesses. The design and frequency of a subclinical ketosis monitoring program will depend on the purpose of the program and the frequency of disease within the herd.

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**Table 1.** Use of cowside ketone tests in screening programs for identifying subclinical ketosis.

Test	20% Prevalence			40% Prevalence			60% Prevalence		
	PV <sup>1</sup> +ve	PV <sup>2</sup> -ve	AP <sup>3</sup>	PV +ve	PV -ve	AP	PV +ve	PV -ve	AP
Keto-Test® using 100 µmmol/L	62%	93%	23%	81%	83%	35%	91%	68%	48%
Ketocheck™ (milk)	90%	86%	8%	96%	70%	16%	98%	51%	23%
Urine	38%	100%	53%	62%	100%	65%	78%	100%	76%

<sup>1</sup>PV +ve: Predictive value of a positive test result.

<sup>2</sup>PV -ve: Predictive value of a negative test result.

<sup>3</sup>Apparent prevalence.



## Nutritional Strategies to Promote Calf Health During the Liquid Feeding Period

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### Abstract

Morbidity and mortality of pre-weaned dairy calves remains a significant problem in the United States. Appropriate nutrition is a critically important component of optimal health for young dairy calves. However, good nutrition cannot overcome the negative effects of poor environmental conditions and large pathogen loads. While no nutritional “magic bullets” exist, improving nutrition of calves in the overall context of good management can be expected to benefit health. Perhaps, the biggest window for improvement lies in improving energy and protein supply to calves during the first two to three weeks of life. Considerable evidence in calves and in other species points to the detrimental effects of inadequate milk intake on susceptibility to disease or impaired function of the immune system. Many feed ingredients and additives are available that have been purported to improve health in calves. The evidence for efficacy of these materials is briefly reviewed in this paper.

### Introduction

Other than the period of transition from pregnancy to lactation, the milk-feeding period for calves represents the time of life when dairy animals are subject to the greatest number of health problems. According to results of the recently released Dairy 2002 survey from the USDA-APHIS National Animal Health

Monitoring System (NAHMS), 8.7% of heifer calves born alive died before weaning. Scouring and other digestive problems were the reported causes of death in 62.1% of reported calf deaths, with respiratory problems representing 21.3%. It is frustrating that these figures have changed little since the 1992 NAHMS survey (NAHMS, 1993), despite increased knowledge and educational efforts on the importance of appropriate colostrum management, nutrition, and health programs. While many dairy farmers achieve much lower calf mortality, these survey results indicate that health of the pre-weaned dairy calf is still a limiting factor on far too many U.S. dairy farms.

Commercial and producer interests in the young calf seem to have increased recently. Several factors may be involved in this renewed interest. Expansion of contract or custom rearing of heifers increases the importance of healthy, well-grown calves. The trend to larger and larger dairy operations has created new issues of practicality in management and places new emphasis on overall profitability. Implications and applications of “accelerated growth” schemes have drawn considerable interest. Perhaps the most important driver in the renewed interest in calf nutrition and management has been the high demand, short supply, and resultant sky-rocketing prices for pregnant heifers during the preceding two calendar years.

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The authors of the NAHMS Dairy 2002 report concluded their summary of results on mortality of U.S. dairy cattle as follows: “Management practices that reduce both calf and cow loss during or immediately subsequent to calving should be reviewed.” (NAHMS, 2002). Given the sobering statistics on the lack of progress in lowering calf mortality, and the renewed interest in practices that may contribute to improved health of dairy calves, the objective of this paper is to address some aspects of nutritional management during the liquid feeding period that may lead to improved calf health.

Calf health resides at the center of complex interactions among the environment, disease-causing agents, and nutrition of the calf (Davis and Drackley, 1998). Breakdowns or deficiencies of management that provide suboptimal environment (lack of sanitation, temperature or humidity stressors, inadequate health management protocols, etc.), poor colostrum management (Davis and Drackley, 1998; Quigley and Drewry, 1998), or suboptimal nutrition may predispose calves to disease. Management must strive to minimize exposure to high populations of disease-causing microbes and maximize the ability of the calf to ward off infection. Success in these areas is often deceptively simple and centers on doing the basic tasks well. Dairy producers, and agribusiness professionals that work with producers, must not be misled into the promise of any “magic bullet” vaccine or nutritional supplement that will overcome poor management.

## **Nutrition and Health**

### *Energy and protein supply*

The biggest opportunity to improve calf health nutritionally may lie in improving the early nutritional status of young dairy calves. Unequivocal data exist for the importance of adequate energy and protein intakes in young animals of many other species, including

humans. There is little reason to suspect that dairy calves would be fundamentally different in this regard.

Conventional heifer calf rearing schemes rely on restricted feeding of milk replacer to encourage early intake of starter. Typical liquid feeding rates of 8 to 10% of body weight are much lower than ad libitum feeding rates. Calves allowed to suckle their dams typically nurse between 6 and 10 times daily and consume between 16 and 24% of body weight as milk (Hafez and Lineweaver, 1968). This translates to 16 to 24 lb, or 1.9 to 2.8 gal, for a 100-lb calf. Since whole milk contains about 12.5% solids, nursing calves would consume 2 to 3 lb of dry milk solids daily and could gain from 2.1 to 3 lb/day. Recent experiments by Dan Weary’s research group at the University of British Columbia have demonstrated that calves of “modern” Canadian Holstein genetics consumed up to 20 lb of milk daily by day 5 of life, and over 22 lb/day by the fourth week of life (Appleby et al., 2001; Jasper and Weary, 2002). Considering standard practice in the beef cattle industry, these facts should not be surprising. Consequently, what is currently termed “accelerated calf growth” probably is more correctly thought of as biologically “normal” growth for young calves.

The focus on the restricted feeding of the liquid diet is to encourage early development of calf starter intake. When management of early weaning programs is excellent, gains of 2 lb/day or more are possible by four weeks of age (Kertz et al., 1979). However, nutrient supplies during the first two weeks of life before starter intake becomes appreciable are very low relative to voluntary consumption rates. Consequently, until starter intake begins to increase late in the second week of life (under the best management in early weaning systems), calves receive enough nutrients from milk or milk replacer only to cover maintenance and low rates of growth.

These concepts are illustrated in Table 1, in which nutrient requirements as specified by the National Research Council (NRC, 2001) are shown. The overall feeding rate determines energy intake and sets limits on the growth possible. Note that as average daily gain (ADG) increases, the required metabolizable energy (ME) intake also increases and more milk replacer powder must be fed to provide that energy. The basis that this idea holds true in practice is demonstrated by recent growth experiments in which calf growth rates increased in direct proportion to the amount of milk or milk replacer fed (Bartlett et al., 2001; Diaz et al., 2001; Jasper and Weary, 2002). Calves require over 0.8 lb of milk replacer powder just to support maintenance; consequently, standard restricted feeding rates that provide only 1 lb of milk replacer powder provides only enough energy to support maintenance and about 0.25 lb of body weight gain daily. It must be pointed out that these requirements assume that calves are housed under thermoneutral conditions; if calves are subject to either heat stress or cold stress, energy intake may be insufficient even for maintenance.

The amount of protein required is largely driven by the rate of growth, because maintenance requirements are small and in theory could be met with as little as 8.3% crude protein (CP) in milk replacer (NRC, 2001; Table 1). Tissue deposition requires an average of 30 grams of nitrogen per kilogram of liveweight gained, or 187.5 g CP per kilogram of ADG (NRC, 2001). Consequently, the amount of CP required by the calf increases as it is fed more energy and rates of gain increase (Table 1). Note in Table 1 that the content of CP needed in milk replacer approaches a maximum in the range of 28% CP.

Also note in Table 1 that the calculated content of CP in milk replacer needed for calves gaining 0.5 lb/day is about 18%. On the basis of calculated protein required for maintenance and growth of calves fed typically recommended

amounts of milk replacer (1 lb/day of powder or 10% of BW), 18% CP likely is sufficient to meet absolute requirements for maintenance and growth. However, increasing milk replacer protein content results in marked increases in rate of gain and efficiency of gain (Donnelly and Hutton, 1976a,b; Bartlett et al., 2001; Blome et al., 2003).

#### *Effects of energy and protein supply on health and immune status*

What is the evidence that inadequate nutrition during early life decreases resistance to disease and compromises health and well being of calves? Williams et al. (1981) compared calves fed two amounts of milk replacer solids (1.3 and either 0.7 or 0.9 lb/day) with either ad libitum or restricted access to calf starter. Calves fed the higher amount of milk replacer with ad libitum access to starter had the greatest ADG and least mortality. Other studies have shown that inadequate nutrition results in impaired immune responses in young calves. Griebel et al. (1987) fed neonatal calves either below maintenance or above maintenance intakes of milk replacer. Calves fed below maintenance lost BW; lymphocytes isolated from these calves had decreased proliferative responses compared with adequately fed calves. Malnourished calves had lower primary antibody response to administration of K99 antigen. Pollock et al. (1993, 1994) compared effects of weaning age (5, 9, or 13 wk of age) and two levels of nutrition (0.9 lb or 2.2 lb/day of milk replacer powder). Weaning at 5 wk resulted in compromised lymphocyte responses (cellular immunity) at 10 wk of age. The higher level of nutrition, which was approximately twice maintenance, resulted in improved responses of cell-mediated immunity and decreased skin responses to antigen (Pollock et al., 1993). In contrast, the high level of nutrition resulted in decreased antibody titers to specific antigens, without changing total Ig concentration in serum (Pollock et al., 1994). More recent evidence demonstrated that feeding for greater (normal)



growth increased nitric oxide and interferon- $\gamma$  production by isolated mononuclear leukocytes compared with cells from conventionally fed calves (Nonnecke et al., 2000).

One way in which improved neonatal nutritional status might be expected to impact the immune system is via growth hormone (**GH**) and the insulin-like growth factors (**IGF**). These anabolic hormones play a direct role in integrating the growth, maintenance, repair, and function of the immune system (Clark, 1997). Lymphocytes express receptors for both GH and IGF-I. In rodents, IGF-I causes growth and maturation of B cells, increases size of the thymus and spleen, and increases antibody production by B cells (see review by Clark, 1997). Consequently, assuming that responses in calves are similar, increased concentrations of IGF-I resulting from improved nutrition might be expected to enhance immunocompetence in calves.

Smith et al. (2002) recently reported that calves fed on an accelerated growth scheme had greater concentrations of IGF-I than did calves fed milk replacer at a conventional (restricted) rate. Plasma from calves in both groups showed increased IGF-I concentrations in response to injection of bovine somatotropin at 5 wk of age, but calves on the higher plane of nutrition also responded with increased growth rates. In our own experiments, IGF-I in plasma (analyzed by M. J. VandeHaar, Michigan State University) was increased by greater feeding rates and increased linearly as CP was increased (data not shown). These results clearly show a functional IGF-I system in young calves that is responsive to early nutritional status. The relationship of the enhanced IGF-1 status to immune function remains to be determined.

A major argument in favor of restricted liquid feeding and early weaning has been that scouring is decreased. In most species, fecal consistency clearly becomes less fluid as dry feed is consumed, primarily as a result of the bulking

effect of dietary fiber. However, merely feeding more milk or more of a high-quality milk replacer does not cause scouring (Mylrea, 1966; Huber et al., 1984; Nocek and Braund, 1986; Appleby et al., 2001; Diaz et al., 2001; Weary and Jasper, 2002). The occurrence of calf scours, unless a poor-quality milk replacer containing damaged ingredients is fed, depends more on the load of pathogenic microorganisms in the calf's environment (Roy, 1980). Our own experiences with calves fed milk replacer at up to 18% of BW indicates that average fecal scores are only slightly (nonsignificant) increased but that the number of days with more fluid fecal scores is increased (Bartlett et al., 2000, unpublished data). Feeding milk replacer results in more fluid feces than feeding similar amounts of whole milk, regardless of the composition of the milk replacer (Bartlett et al., 2000, unpublished data).

Lessons from other species also should be useful for improving our management of dairy calves. The degree of immune challenge presented by the environment can have marked effects on growth and feed efficiency. For example, pigs subjected to challenge with bacterial lipopolysaccharide had decreased growth and feed efficiency compared with control pigs; growth for challenged pigs was lower than pair-fed littermates, indicating that decreased feed intake accounted for only about 2/3 of the growth depression (Dritz et al., 1996). Effects of immune challenge did not interact with diet complexity (Dritz et al., 1996). Pigs raised in a clean environment with minimal exposure to pathogens had greater rates of gain, improved feed efficiencies, and a greater lean-to-fat ratio than pigs raised in the presence of a high degree of pathogen exposure (Williams et al., 1997). Such responses are probably attributable both to increased nutrient demands by the immune system and to the anti-growth effects of cytokines produced by the activated immune system. Klasing and Calvert (1999) calculated that up to 60% of the impaired growth of chicks during an intense immune response

could be explained by known processes. Almost 7% of lysine intake was used by immune processes during a lipopolysaccharide challenge in chicks (Klasing and Calvert, 1999). Consequently, nutrient demands of the immune system may compete directly with growth processes for a limited supply of nutrients.

A fascinating discussion of the partitioning of nutrients between maintenance, immune function, and growth or other productive functions has been provided by Houdijk et al. (2001). In particular, the possibility that inadequate protein supply may limit function of the immune system in calves is intriguing. The potential importance of protein is suggested indirectly from our own experiments (Bartlett et al., 2001; Blome et al., 2003) in which increasing protein content of isocaloric milk replacers markedly increased ADG and efficiency of gain. For example, in limit-fed calves (12% of body weight as liquid) offered isocaloric milk replacers in which the protein content increased from 18% to 26%, ADG (from 0.84 to 1.36 lb/day) and efficiency of gain (from 0.51 to 0.78 lb gain/lb feed) increased linearly at similar overall energy retention (Blome et al., 2003).

#### *Vitamins and trace minerals*

Many of the vitamins and trace minerals can impact function of the immune system in calves. For a detailed discussion of these effects in dairy cattle, readers are referred to the NRC (2001). Vitamin E deserves special mention here. A series of experiments by Jim Morrill and colleagues at Kansas State University (Reddy et al., 1986, 1987a,b; Eicher-Pruett et al., 1992; Eicher et al., 1994) has built a convincing argument that vitamin E is beneficial to calf health. The latest NRC committee modestly raised the requirement for vitamin E from 18 IU/lb to 23 IU/lb of dietary dry matter (NRC, 2001). The committee declined to raise the vitamin E requirement further in the absence of large clinical studies demonstrating health

benefits. Most manufacturers of milk replacers routinely supplement with much higher amounts of vitamin E.

#### **Type of Liquid Feed**

Options for feeding after colostrum include whole milk, excess colostrum and transition milk, waste or discard milk, and milk replacer. Pros and cons associated with each feed type have been discussed in detail elsewhere (Davis and Drackley, 1998; Drackley, 1999). Many producers successfully use a pool of all non-saleable milk to feed calves. Concerns about bacterial load have resulted in increases in on-farm pasteurization on larger farms. California studies (Jamaluddin et al., 1996a,b) showed that pasteurization increased growth rates of calves and that calves fed pasteurized non-saleable milk were worth \$8.13 more than calves fed non-pasteurized milk. While proper pasteurization is effective in inactivating the causative bacteria for Johne's disease (Stabel, 2001) and *Mycoplasma mastitis* (Butler et al., 2000), it will not destroy all viruses. Producers in strict biosecurity programs should feed milk replacer.

Milk replacers are fed on a majority of U.S. dairy farms (Heinrichs et al., 1995). High-quality milk replacers are excellent liquid feeds for young calves. Because of the high cost of dried skim milk, the all-milk protein milk replacers used currently are based almost exclusively on whey proteins (whey protein concentrate, dried whey, and delactosed whey). Milk replacers based on whey proteins are digested and utilized at least as efficiently as the skim milk proteins used in earlier milk replacer formulations (Terosky et al., 1997; Lammers et al., 1998). Reports of poor calf performance on milk replacer often are attributable to selection of an inappropriate or poor-quality milk replacer, to underfeeding the calf, or to an underlying disease or sanitation problem. Milk replacer almost always will be a cheaper feed for young calves than saleable whole milk. Although more

expensive than surplus colostrum, transition milk, or waste milk, milk replacers have advantages in consistency of product from day to day, ease and flexibility of storage, and disease control. An in-depth discussion of the manufacture and use of milk replacers can be found elsewhere (Davis and Drackley, 1998).

Waste or discard milk and excess colostrum/transition milk are often thought of as “free feeds”. However, if waste milk were not being produced, then the “free milk” would be receiving the milk sale price. Thus, there is significant “opportunity cost” associated with excessive dumping of milk, which emphasizes one of the many reasons for mastitis control to increase profits to dairy producers. Nevertheless, nearly all farms will have some waste milk available at times. On the other hand, excess colostrum and transition milk has no other use – it is truly a “free feed” except for additional labor costs and costs associated with storage. However, producers must keep in mind the disease control considerations of feeding any waste milk or colostrum/transition milk, which could significantly increase the “true” costs of using these feeds.

Many producers will use whatever non-saleable milk is available each day to feed calves, whether excess colostrum, transition milk, or discard milk. This practice results in the calf receiving a diet that varies considerably in composition from day to day. Such variability reportedly does not affect the incidence or severity of scouring or overall rates of gain (Foley and Otterby, 1978). Even frequent changes between sources of colostrum or waste milk and milk replacer did not affect calves adversely in several earlier studies (Appleman and Owen, 1975). However, maintaining as much consistency as possible in the diet for young calves minimizes chances for digestive upsets. This may be particularly important when calves are raised under conditions of increased stress, such as cold or wet weather or during outbreaks of disease.

## Effects of Additives and Supplements on Calf Health

### *General perspectives*

As mentioned in the introduction, the likelihood of finding a “magic bullet” supplement for improved health in calves is remote. Nevertheless, supplements may be helpful in improving health or growth of calves. One of the problems in evaluating the current literature on such additives is that most have been tested in calves fed at restricted intake, and thus have been more likely limited by energy and protein intakes than by the ingredients in the additives. The usefulness of additives may be enhanced if tested against the background of more adequate nutrition. For many of these additives, biologically significant increments in performance or improved health may be too small to detect statistically in many experiments because of inadequate numbers of calves.

It also is possible that several of the following components may each provide small improvements that will be additive, such that a product combining several different types of compounds might show substantial efficacy. One hope is that such combinations might be able to compensate for antibiotics if use in livestock feeding is eventually banned in the U.S. For example, a product containing probiotics, fructooligosaccharides, and allium supplemented to milk replacer without antibiotics provided similar calf performance and health as a medicated milk replacer (Donovan et al., 2002).

### *Antibiotics*

The use of antibiotics in all animal production is coming under increasing criticism. However, antibiotics typically added to “medicated” milk replacers (primarily oxytetracycline and neomycin) still are clearly effective in improving performance and health of calves (Morrill et al., 1977b; Quigley et al.,

1997; Davis and Drackley, 1998). For producers with excellent sanitation and good nutrition programs, medicated milk replacers probably are not necessary. However, under less than optimal management, or where producers are bringing calves from other farms or unknown sources on their farm, medicated milk replacers will almost always be beneficial (Davis and Drackley, 1998).

### *Coccidiostats*

Coccidiosis is a common clinical or subclinical cause of poor performance, illness, and economic loss in dairy calves. Use of coccidiostats (decoquinate or lasalocid) in milk or milk replacer has been shown experimentally to lessen the effects of coccidiosis and improve gains (Bauer et al., 1992; Webb et al., 1992; Quigley et al., 1997a). While use of coccidiostats in starters has been widely recommended and adopted, calves may not consume sufficient amounts of starter before 4 wk to prevent infection during the first 2 to 3 weeks of life (Quigley et al., 1997a).

### *Prebiotics and Probiotics*

Prebiotics are live bacterial cultures designed to increase colonization of the digestive tract with species that produce favorable effects and compete with pathogenic microorganisms for nutrients and attachment sites. Various prebiotic bacterial cultures have been examined and promoted for use in dairy calves. A combination of *Bifidobacterium pseudolongum* and *Lactobacillus acidophilus* in the diet of pre-weaned calves increased body weight gain and decreased scouring (Abe et al., 1995). Calves fed a probiotic (composition not reported) without antibiotics had growth rates and health measures similar to that of calves fed a similar milk replacer with antibiotics (Morrill et al., 1995). Small but generally positive effects from bacterial probiotics have been noted in other studies (Jenny et al., 1991; Higginbotham and Bath, 1993; Cruywagen et al., 1996).

Prebiotics are compounds that are indigestible by the host animal's digestive enzymes but are usable by microorganisms. These compounds provide substrate for growth of "beneficial" bacteria in the intestine or compete with pathogenic bacteria for intestinal attachment sites. Complex carbohydrates such as oligofructose, mannanoligosaccharides, and others have shown promise in young calves as well as other species (Webb et al., 1992; Kaufold et al., 2000; Flickinger and Fahey, 2002). Calves fed the trisaccharide galactosyl-lactose in milk replacer had greater weight gains and fewer days scouring than control calves (Quigley et al., 1997b).

### *Plasma proteins and immunoglobulins*

Plasma protein products have been investigated for their potential to increase growth and health of young calves as they do in young pigs (e.g., Hansen et al., 1993). Calves fed milk replacers containing plasma protein had similar (Quigley and Bernard, 1996) or improved performance (Morrill et al., 1995; Quigley et al., 2002) relative to controls. Supplements prepared from serum or immunoglobulin fractions also may have promise for improving calf health (Quigley and Drew, 2000; Quigley et al., 2002).

### *Other additives*

Many other compounds have been purported to have health benefits. A recent study documented improvements in health and growth from addition of fresh or autoclaved rumen fluid to young calves' diets (Muscato et al., 2002). The mode of action for the effect is uncertain but may be related to stimulation of the immune system by bacterial polysaccharides present in the rumen fluid extracts. There is considerable interest in other "nutraceutical" compounds for calves, such as garlic extracts or other herbal products. For example, allicin, a component of garlic, has antimicrobial properties against a variety of microbial classes. A clinical trial demonstrated that allicin had little effect on the

infection by *Cryptosporidia* in young calves (Olson et al., 1998). There are relatively few reports in refereed scientific journals on the efficacy of herbal products in calves.

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**Table 1.** Effect of rate of body weight gain with constant initial body weight (100 lb) on protein requirements of pre-weaned dairy calves (adapted from National Research Council, 2001).<sup>1</sup>

Rate of gain (lb/day)	ME (Mcal/day)	ADP (g/day)	Required DMI <sup>2</sup> (lb/day)	CP Required (% of DM)
0	1748	28	0.84	8.3
0.50	2296	82	1.11	18.1
1.00	3008	136	1.45	22.9
1.50	3798	189	1.83	25.3
2.00	4643	243	2.24	26.6
2.50	5532	297	2.67	27.2

<sup>1</sup>ME = metabolizable energy, ADP = apparently digestible protein, DMI = dry matter intake, CP = crude protein, and DM = dry matter.

<sup>2</sup>Amount of milk replacer DM containing 2075 kcal ME/lb DM needed to meet ME requirements.

# Effects of Mycotoxins on Ruminal Bacteria and Animal Performance

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## Abstract

Dairy producers have a difficult task when attempting to assess the possible exposure of their animals to mycotoxins. Information gleaned from the scientific literature suggests signs and symptoms for toxins which may actually not be likely to appear in a herd. Also, that same literature suggests concentrations at which the toxin exerts its effects. Those concentrations may have little meaning in a production setting in which multiple mycotoxins, as well as other production stresses, are at work. We do know, however, that mycotoxins do pass through the dairy cow's rumen intact, although clearly some microbial metabolism can and does take place. At the same time, the mycotoxins may produce adverse effects on key rumen bacteria. Therefore, we cannot count on rumen microbiology to protect the dairy animal. Predicting signs which might occur in the herd exposed to mycotoxins is also futile. Such signs are generally not unique to particular mycotoxins if to toxic compounds at all. Changes in productivity, in reproductive capacity, in general health, behavior, etc. may all signal the presence of mycotoxins, although other causes may be equally to blame. The present report discusses some of these factors and suggests the approach dairy farmers may find most productive in dealing with the issue of mycotoxin contamination on their farms.

## Introduction

Case study reports and research trials can provide both valuable and misleading clues about the effects of mycotoxins in ruminants. A herd experienced a decrease in breeding efficiency over an extended period (~ five months), with reduced birth weights, unhealthy calves, increased mastitis, prolapsed rectum, decreased feed consumption, and other signs. Corn contaminated with aflatoxin at up to 120 ppb had been included in the ration. Once the contaminated material was found and removed, milk production rose by 28% (Guthrie, 1979). Choudary et al. (1998) reported that as little as 10 ppb of aflatoxin B<sub>1</sub> (**AFB**) resulted in a significant reduction in feed consumption. Also responding in a dose-dependent fashion, rumen motility declined in dairy cows receiving 200 to 800 ppb AFB (Cook et al., 1986). Of course, many producers throughout the U.S. and other regulated production areas understand the loss that can occur when violative levels of aflatoxin M<sub>1</sub> are discovered in milk.

Calves consuming the cyto-necrotic T-2 toxin (at 10 ppm and above) developed ulcers of the abomasum and sloughing of rumen papillae (Cheeke, 1998). Goats fed 95 ppm fumonisin showed no overt signs of toxicosis although sphingolipid tests revealed toxin-related pathology (Gurung, et al. 1998), but dairy cows fed that toxin did not present either circulating fumonisin or alterations in

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sphinganine/sphingosine ratios (Prelusky et al., 1995). When fumonisin was administered to Holstein calves, liver and renal toxicity with multiple signs resulted (Mathur et al., 2001); however, these were young calves that had not yet developed rumen function. So, are fungal toxins a threat to dairy operations?

Many have suggested that the bacteria of the bovine rumen are exceptionally efficient at degrading dietary mycotoxins. Once destroyed, therefore, there is no opportunity for these compounds to be absorbed and exert their toxic roles. Swanson et al. (1987) demonstrated that when **DAS** (diacetoxyscirpenol), **DON** (deoxynivalenol), and T-2 toxin were incubated with rumen fluid, all three were rapidly metabolized by the microflora. However, end products of that 'degradation' process were identified. Many of them, such as monoacetoxyscirpenol (**MAS**) are also toxic compounds. Earlier, Kiessling et al. (1984) had reported that rumen contents (fluid and microflora) would metabolize ochratoxin, zearlanenone, DAS, and T-2 toxin but not DON or AFB (in vitro).

Dairy farmers, therefore, face some significant dilemmas. First, some of the reported signs are relatively non-descript and do not consistently pertain to specific mycotoxin exposures. Diarrhea, lowered feed consumption, mastitis, estrus errors, etc. may all be part of one or more mycotoxins' impact on the dairy animal; however, none are unique to fungal toxins and most may occur more often as a result of other factors. Similarly, if one takes the research on ruminal degradation of mycotoxins at first blush, it would seem that ruminants are uniquely protected against these fungal metabolites. How, then, can it be that staggers, slobbers, abortion, loss of production, hypothermia, etc. can all be induced by a dietary application of certain toxins at levels consistent with what is found naturally under farm conditions?

## Factors Associated with Rumen Function

### *Mycotoxin metabolism by ruminal bacteria*

The literature on microbial degradation of various mycotoxins by bacteria is abundant, and begins, generally, with evaluation of common soil microorganisms. However, of interest are those studies dealing with either mixtures or isolated species of organisms taken from biological sources. Galtier and Alvinerie (1976) isolated cecal contents from rats and demonstrated that ochratoxin A could be degraded to the non-toxic alpha form by the microflora but not by the supernatant fluid of a centrifugal separation. Thus, the live bacteria were considered responsible for the activity. They also showed that bovine or ovine rumen fluid metabolized ochratoxin A but at times esterified it to ochratoxin C which is just as toxic as A. The rumen fluid, however, was obtained at a slaughter house; fluid obtained via esophagus did not convert ochratoxin A to C and did hydrolyze it to the alpha form (Galtier and Alvinerie, 1976). They suggested that this effect was mediated by protozoa rather than bacteria. This also, then, suggests that in vitro studies may be influenced greatly by the means by which ruminal fluid/contents are obtained. Post-mortem versus esophageal aspirates versus fistula samples must be considered very different test materials. He et al. (1992) suggested from their studies with soil, chicken and swine intestinal, and rumen fluid bacteria that chemical modification of DON was influenced by several conditions. Thirty-five percent of the original DON was metabolized by rumen fluid; however, lowering the pH of the test medium fully inhibited this metabolism. Further, they determined that while deepoxy DON (the de-epoxidation product) was the major metabolite, it was highly dependent on energy availability (He et al., 1992). Does this have implications for studies in which rumen fluid and microflora have been separated from ingesta before being used for metabolism tests?

Xiao et al. (1991) measured hydrolysis of ochratoxin A by ovine rumen. Both in vitro (hydrolysis product) and in vivo (disappearance half-life) metabolism of ochratoxin A were found to be influenced by the diet of the sheep. Grain-fed sheep did not metabolize the toxin nearly so well as those fed hay. Their explanation was simple and logical: differences in diet result in differences in the microbial profile of the rumen. Thus, the question of whether a toxin may be degraded or not, much less the apparent rate or efficiency of that degradation, is subject to the specifics of the microflora resident at the time the rumen material is collected. In turn, that is strongly driven by what the donor animal has consumed prior to that sampling. Muller et al. (2001) have recently confirmed this effect using cows fed diets with grass, grass silage or hay, and different amounts of a concentrate (barley/soymeal). In addition to dietary effects, they also found that the animal, itself, affected the results.

Many studies could be cited. However, the essential points are that 1) many bacteria and other microorganisms are capable of assimilating and metabolizing mycotoxins (or other xenobiotics). These include species found in the normal, functional microbiota of the bovine rumen. At the same time, the relative efficiencies of such organisms to achieve full success under a wide ranging variety of conditions and circumstances are very unclear. It is important not to assume that metabolism of a compound means that all of that compound is now accounted for. Many reports indicate that only some percentage of the total compound tested is really changed. 2) Bacteria found to metabolize a foreign compound under one set of experimental conditions may not do so under another. 3) In vitro is just that! By its very nature, the test is artificial and subject to many influences not accounted for in all studies. That is not to say that in vitro work is not a good idea; much useful information is provided, but direct extrapolation to the effects that might occur in the intact rumen of a production unit animal are, at best, potentially wrong, and, at

worst, dangerous to the extent that others interpret such results as real.

#### *Mycotoxin effects on ruminal bacteria*

While it is important to understand that at least some change in ingested mycotoxins is probable in the rumen, it is of equal importance to understand that the reverse is true. Rumen microorganisms can be adversely impacted by mycotoxins. Periodically, there is interest in the condition known as salivary syndrome (or slobbers) caused by infestation of clover by *Rhizoctonia leguminicola* and its resultant toxin, slaframine. Froetschel et al. (1986) showed that rumen contractions decreased from 20 to 78% with the introduction of slaframine into the rumen of sheep and cows. Later, the same group (Froetschel et al., 1987) reported that total ruminal fluid volume and outflow increased by 25% or more in response to slaframine. Ruminal digestion of DM, ADF, and starch declined while post-ruminal digestion of those same components increased (Froetschel et al., 1989). Bird et al. (1993), working with sheep, obtained similar results with respect to slaframine-induced fluid volumes and reported an increase in cellulolytic bacteria; however, their study had the additional factors of diet variables (low-quality hay with or without cottonseed meal) and found the meal supplementation essential to counter-act weight loss associated with the toxin.

Escoula (1992) found that patulin from laboratory cultures reduced acetate production when added to in vitro rumen fluid. Further, protein synthesis was inhibited in that same system by the toxin in a dose-dependent fashion. Patulin is most often associated with bruised fruits, especially apples; therefore, it is unlikely to be of major significance to most dairy herds except in areas where apple pressing residues (e.g., for fruit wines or ciders) are used in animal rations. Earlier, reference was made to Cook's study (Cook et al., 1986) involving graded doses of AFB and slowing of rumen motility. However, they also noted that because of that

change in motility, the time required to eliminate AFB from rumen contents increased, a feature they found helpful as a diagnostic aid. However, it must also be considered that two additional circumstances may occur because of this longer in rumen residence time for the xenobiotic. On the one hand, there is greater opportunity for capable bacteria to metabolize more of the toxin. On the other hand, however, that same time may allow AFB or other toxins to have a greater negative impact on specific members of the rumen microflora.

Arguably, dairy cows consume more *Fusarium* toxins than aflatoxins. Recently, May et al. (2000) published data indicating that fusaric acid was inhibitory to two different rumen organisms, *Methanobrevibacter ruminantium* (an end-of-fermentation methane generator) and *Ruminococcus albus* (an onset-of-fermentation cellulolytic bacterium). Low levels of fusaric acid (e.g., ~7 ppm) were sufficient to affect microbial growth; at high levels (e.g., ~ 60 ppm), growth stopped altogether. Further, upon removal of the toxin, neither organism recovered. These two bacteria represent quite different evolutionary positions. *R. albus* is a true prokaryote; *M. ruminantium* has both pro- and eucaryotic biochemistry. For fusaric acid to inhibit both suggests that it may work in more than one way mechanistically; that is, it expressed its toxicity in one by a route different than in the other. If this is proven true, it presents a new dilemma in that multiple modes of actions increase the likelihood and avenues by which this toxin might interact with other mycotoxins. Addition of DON to tests in this work (May et al., 2000), if anything, spared the fusaric acid effects on the two bacteria. Can we predict a similar response if zearalenone, or AFB, or slaframine is present?

### **Mycotoxin Effects on Dairy Cattle**

Field cases generally present with non-descript signs, including reduced feed

consumption and milk production, lower fat and/or protein in milk, reproductive ‘errors’ (altered cycles, etc.), and to more dire conditions including abortion, morbidity, and death. As farmers note the onset of such signs, there is generally a systematic attempt to resolve the underlying cause(s) and determine a solution. In acute mycotoxicotic episodes, by the time the veterinarian, nutritionist, breeder, etc. have completed examination, the particular feedstuff responsible may well be gone. More typically, however, chronic exposure to multiple mycotoxins underlies the problem with large animals. Not too many years ago, I might have looked first at grain supplements, cottonseed meal, and other by-products used in feeding as the first choice of possible contaminated products. That, of course, stemmed from the common misconception that since ensiled materials are in an anaerobic state, they cannot support active mold growth (or mycotoxin production). However, silage is most often the first place we should look.

Points to keep in mind about silage are simply these: 1) structures in which ensiling takes place and in which the product is stored until fed are not necessarily as air-tight as we would like to suppose. 2) Some of the mycotoxigenic molds are micro-aerophilic; that is, they grow under very low oxygen tension. Ensiling may alter conditions to reduce some genera (e.g., *Aspergillus*) but produce a competitive advantage for others (e.g., *Penicillium*). 3) Some molds, again often in the genus *Penicillium*, actively metabolize low molecular weight organic acids (e.g., propionic acid) that may be used initially to defeat molds until anaerobiosis is achieved or that may form as a result of bacterial fermentation action during ensilation (e.g., acetic, et al.). 4) The mechanical action of cutting/removing silage for feeding can introduce fresh air into the next ‘segment’ of silage. For toxigenic molds that are inactive prior to that new infusion of oxygen, the response tends to be rapid – significant amounts of toxins

may be synthesized within 24 to 48 hours. At the next feeding, cows are presented with newly contaminated silage.

Analysis of silage is not an easy task. Obtaining representation in the sample and appropriately preparing the matrix for extraction are both major hurdles for the dairy farmer that other animal producers (e.g., poultry) do not face. The coarse, highly heterogeneous nature of silage requires special preparation for testing. Molds, while often seen as surface contaminants, tend not to be surface metabolizers in feedstuffs. The corn kernel represents a good example. We often see mold spores or even visible mold on the surface; however, few molds gain much from the pericarp. Instead, they penetrate and metabolize the starch portion of the kernel. If one tries to extract such corn with a relatively benign solvent system, such as aqueous methanol (which is the extraction solvent required for most rapid antibody-based tests), much of the mycotoxin will never be extracted since the waxy exterior of the kernel retards penetration by the solvent. In order for the test to work, kernels must be ground to a very fine particle size (generally small enough to pass a 20-mesh sieve). One can make the same provisions for silage, except with much greater difficulty than with corn. Since virtually all of the toxins of importance are relatively heat stable, silage samples can be dried and then ground through a conventional laboratory milling system. However, for those attempting on-farm analyses, maintaining a heavy duty blender with a sharp blade assembly can allow one to comminute the sample within the extraction solvent system. The key to success here, which means getting sufficient amounts of analytes extracted that some reasonable estimate of actual condition of the feedstuff is possible, is processing an adequate amount of sample in enough solvent to do the job. That may well mean 'scaling up' from what a company selling rapid tests suggests.

Farmers reporting signs and having gotten beyond the issues of infectious disease, nutritional error, etc. are then faced with how to interpret a sample analysis. A typical result may show a silage sample containing 600 ppb zearalenone, 1.8 ppm DON, 26 ppb aflatoxin, positive for fumonisin, and perhaps a little DAS in the mix for good measure! Cows are off feed, milk production is down, and somatic cell counts are up. Which sign goes with which mycotoxin? Or do any of the problems match any of the toxins at all?

This report opened with the concept that both valuable and misleading information abounds. Research reports on controlled single mycotoxin experiments in a few animals generally do a very good job with the descriptive toxicology of a particular mycotoxin under a special set of experimental conditions. One can learn valuable information about how the toxin works and what may be the results of exposure to that toxin. At the same time, the test animals in most research trials are relatively protected in that an investigator is trying to isolate their treatments as the sole causes of ultimate results. So, the kinds of management, nutritional, production, behavioral, environmental, etc. pressures faced by the animal on the farm are less a factor. Yet, these all impact how that animal responds to a single mycotoxin. The real problem is what we find in that typical case mentioned just above - multiple toxins. How do we interpret such a result?

First, we must allow for the fact that many of our tests are precise but not necessarily accurate in the field. So, the absolute values in ppm or ppb provide a frame of reference but not a number that we should be overly enthused about. Second, the finding of one or more toxins tells us that conditions have favored the active growth and metabolism of toxigenic molds. Since there are hundreds of species and several hundred different mycotoxins, our positive tests should alert us to the real possibility that our feedstuffs contain much more than those four

or five test results tell us. Third, the toxins we select for testing are often those which, while important themselves, are relatively easy to assay and may reflect a “family” of related chemical compounds. Thus, measuring DON not only tells us about toxin specifically, but also indicates that we should expect other mycotoxins of the trichothecene class to be present. Finally, and most importantly, the finding of more than one toxin opens the sure possibility that chemical interactions are at work.

The idea that mycotoxins can potentiate or antagonize the action of other mycotoxins is no longer a supposition. It has been proven extensively in the literature. However, even with those kinds of scientific studies, we have only looked at a limited number of interaction combinations (e.g., two or maybe three mycotoxins at a time). We have little ability to predict what might happen when four or five or more toxins are present together in the animal’s ration. To further complicate this issue, we now are beginning to understand that a given animal may react to two mycotoxins quite differently depending on which toxin gets in first! Gelderblom et al. (2002) have studied this phenomena in rat liver using fumonisin and aflatoxin. Ultimate pathology was shown to depend on whether the samples were treated with fumonisin first or second. In short, if aflatoxin got to the liver first and caused damage, then the response to the fumonsin changed quite drastically compared to liver treated with fumonisin first (Gelderblom et al., 2002).

Finally, low level chronic exposure to mycotoxins may fail to induce even a single sign that can truly be associated with any given mycotoxin. However, almost universally, mycotoxins impact immune function in animals. Chronic exposure, then, often leads to an increase in opportunistic pathogens gaining a foothold in the animal. Subsequently, we find symptoms associated with infectious disease rather than with an intoxicant. Predicting specific signs, therefore, becomes relatively futile. The

best advice to producers, therefore, is to consider that whenever production and health decline in a herd, mycotoxins should be among the factors considered, and steps should be taken to reduce or eliminate those toxins.

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## Effects of Dietary Fat on Reproduction

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### Abstract

Prostaglandins are active substances synthesized from dietary fat and involved in reproduction. There are two main pathways used to synthesize prostaglandins. One is used by most dietary fat (e. g. corn and soybean, sources of omega-6 fatty acids) and leads to series 1 and 2 prostaglandins, while the other one is more specific to fish products and flax oil (sources of omega-3 fatty acids) and leads to series 3 prostaglandins. Thus, depending on the pathway used for prostaglandins synthesis, the type and role of the resulting prostaglandins will differ. Fatty acid composition of the cell wall membrane is modified by dietary fatty acids, which would alter the function of reproductive tissues. This suggests that fatty acids from different metabolic pathways have different effects on reproduction of the dairy cow. In fact, dietary omega-3 fatty acids can decrease series 2 prostaglandins synthesis by different actions, which include decreasing the availability of the precursor arachidonic acid, increasing the concentration of fatty acids that compete with arachidonic acid for series 2 prostaglandins, and inhibiting prostaglandins synthase. Lower secretion of series 2 prostaglandins usually improves reproduction of cattle. This can be achieved through dietary supplementation with fish oil or linseed oil as they are major inhibitors of desaturation and elongation, leading to arachidonic acid formation.

### Introduction

Recently, there has been a great deal of interest in feeding fat to dairy cows in order to increase energy density of the diet and improve reproduction. It is known that cows fed supplemental fat may experience improved energy balance and begin to cycle sooner because of enhanced follicular growth and development (Grummer and Carroll, 1991). However, Lucy et al. (1992) suggested that it was fatty acids, and not the additional energy provided by the fatty acids, that stimulated ovarian function. Recently, new information has been published that demonstrates that the type of dietary fatty acids is important, as individual fatty acids do not have the same effects on reproduction of the dairy cow.

### Fatty Acid Terminology

A fatty acid molecule is shaped like a caterpillar with two different ends: a methyl group and a water-soluble end that is the carboxyl end. There are different families of fatty acids in feed: omega-3, omega-6, omega-7, and omega-9. The most common numbering system is called the omega system. This system numbers carbon atoms in sequence, starting from the methyl end. The other commonly used system, called the delta ( $\delta$ ) system, starts at the acid end and numbers the carbon atoms in reverse direction.

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The omega-7 family of fatty acids is synthesised from palmitic acid (C16:0), while the omega-9 fatty acid family is synthesised from stearic acid (C18:0) via oleic acid (C18:1, Figure 1). These two families are not considered essential as they are produced in the body. The omega-3 and omega-6 fatty acids are essential because both are vital to health but cannot be made by our cells and must, therefore, be provided by foods.

Linoleic acid (C18:2) belongs to the omega-6 family, while linolenic acid (C18:3) belongs to the omega-3 family (Figure 2). The system used to name fatty acids considers the number of carbons in the chain (e.g. 18 for linoleic acid), the number of double bonds in the chain (2 for linoleic acid), and where in the chain the first double bond is located from the methyl end (1st double bond between carbons 6 and 7 for linoleic acid): C18:2.

### Sources of Fatty Acids

The main sources of short chain fatty acids are cottonseed and palm oils. All sources of fat contain long chain fatty acids. The main sources of linolenic acid (C18:3n-3) are flaxseed, hemp, canola, soybean, nuts, and dark green forages. Ryegrass silage contains as much as 60% of linolenic acid as a percentage of total fatty acids (Dewhurst and King, 1998), which would encourage the use of high forage systems to increase dietary linolenic acid content. Omega-3 fatty acids are found also in cold water and salt water fish (salmon, trout, mackerel, and sardines). The main sources of linoleic acid (C18:2n-6) are sunflower seed, safflower, hemp, soybean, nuts, pumpkin seeds, sesame seeds, and flaxseed. Gamma-linolenic acid (C18:3n-6) is found in evening primrose oil, grape seeds, and borage. Dihomogamma-linolenic acid (C20:3n-6) is found in maternal milk, while arachidonic acid (C20:4n-6) occurs mainly in meat and animal products. Oleic acid (C18:1) is found in olive, almond, avocado, peanut, pecan, cashew, macadamia nut, and butter. Omega 7 in the form of palmitoleic acid (C16:1) is

found in tropical oils (coconut and palm). Composition in C18 fatty acids of some edible vegetable oils is reported in Table 1.

### Fatty Acids and Fertility

Supplementary fats are likely to affect fertility because fatty acids are the precursors both of prostaglandins (PG) and, via cholesterol, the steroid hormones. In general, feeding supplemental fat, such as calcium soaps of long chain fatty acids, fish meal, and tallow, increases conception rates. However, a lowered conception rate at first service has been reported when there was a paralleled increase in milk production (range of 4.8 to 9.9 lb/day; 2.2 to 4.5 kg/day). Thatcher and Staples (2000) wrote an excellent review on the subject. There are two main families of essential fatty acids, omega-3 and omega-6 fatty acids, that could affect fertility. The main source of omega-6 fatty acids is dietary linoleic acid (C18:2n-6) and this is converted to arachidonic acid (C20:4n-6), which *inter alia* is the precursor of the dienoic (2 series) PG, such as  $\text{PGF}_{2\alpha}$ . The same elongase and desaturase enzymes also convert the main dietary omega-3 fatty acids ( $\alpha$ -linolenic acid; C18:3n-3) to eicosapentaenoic acid (EPA; C20:5n-3), the precursor of the trienoic (3-series) PG, such as  $\text{PGF}_{3\alpha}$  (Abayasekara and Wathes, 1999). Competition between omega-3 and omega-6 precursors for desaturation and elongation as well as at the site of PG synthetase means that increasing the supply of omega-3 fatty acids will decrease production of dienoic PG (Barnouin and Chassagne, 1991). In many cases, the trienoic PG have lower biological activity than the corresponding dienoic PG (Fly and Johnston, 1990), and this may directly affect aspects of fertility. For example, treatments that reduce ovarian and endometrial synthesis of  $\text{PGF}_{2\alpha}$  at the expense of  $\text{PGF}_{3\alpha}$  may contribute to a reduction in embryonic mortality (Mattos et al., 2000). There is some evidence for different effects of  $\alpha$ -linolenic acid and the omega-3 fatty acids from fish oil (EPA and docosahexaenoic acid (DHA), C22 :6n-3) on eicosanoid (interleukin)

synthesis, perhaps because of differences in the way in which these fatty acids are incorporated into cell membranes (Wu et al., 1996).

Supplementary fats can also reduce the total synthesis of PG by affecting the activity of PG synthase (Thatcher et al., 1995). Diets rich in linoleic acid (C18:2) increase arachidonic acid concentration (C20:4) in tissues, and diets rich in linolenic acid (C18:3) increase concentration of eicosapentaenoic acid (C20:5) (Béréziat, 1978). Moreover, eicosapentaenoic acid (C20:5) is a competitive inhibitor of the enzyme complex involved in the synthesis of PG from arachidonic acid (C20:4) (Leat and Northrop, 1979). Therefore, this would suggest that a diet with a low linoleic to linolenic acid ratio (C18:2:C18:3, omega-6:omega-3) could decrease PG secretion or PG activity as suggested by Barnouin and Chassagne (1991), which would thus have important effects on reproduction and immunity in the dairy cow.

### Prostaglandin Synthesis

There are two main pathways used to synthesize PG (Figure 3); one is used by most dietary fat (e.g. corn and soybean, sources of omega-6 fatty acids) and leads to series 1 and 2 PG, while the other one is more specific to fish products and flax (sources of omega-3 fatty acids) and leads to series 3 PG. Thus, depending on the pathway used for PG synthesis, the type and role of the resulting PG will differ. The PG of series 2 are important at calving; they increase platelet agglutination and blood clot formation, and they increase salt retention in the kidneys, water retention, and blood pressure. The PG of series 2 also cause inflammation, which lead to their role of “bad guys” among the different PG series. The PG of series 1 improve the immune system of T cells, prevent platelet agglutination and heart attack, contribute to removal of excess Na and water by the kidneys, decrease the inflammatory response, contribute in controlling arthritis, and contribute to decreasing cholesterol production. The PG of the series 3 have a very

weak platelet agglutination power and they prevent fabrication of PG of the series 2; they also prevent heart attack, water retention, and inflammation. The PG of the series 1 and 3 are thus considered as “good guys” contrary to those of the series 2.

Some polyunsaturated fatty acids (PUFA) can serve as a substrate for the synthesis of PGF<sub>2α</sub>. These include cis-linoleic acid (C18:2) that is commonly found in natural fat sources. It can be desaturated and elongated to form arachidonic acid, which serves as an immediate precursor for the series 2 PG of which PGF<sub>2α</sub> is a key member. Key regulatory enzymes for these conversions include Δ6 desaturase and cyclooxygenase. Fatty acids also can inhibit PG synthesis by competitive inhibition with these key enzymes. The EPA and docosahexanoic acid (C22:6) have been shown to inhibit cyclooxygenase activity, which is an enzyme involved in the synthesis of PGF<sub>2α</sub>.

### Fatty Acids, Cholesterol, and Progesterone

Cholesterol serves as a precursor for the synthesis of progesterone by ovarian luteal cells. Secretion of progesterone is the main function of the corpus luteum. Progesterone not only prepares the uterus for implantation of the embryo but also helps maintain pregnancy by providing nourishment to the conceptus. The successful establishment and maintenance of pregnancy (before day 16 post AI) requires the maintenance of progesterone secretion through the critical period of the maternal recognition of pregnancy when luteolysis occurs in the non-pregnant animal (Lamming and Royal, 2001). Between 25 and 55% of mammalian embryos die in early gestation. Increased concentrations of plasma progesterone have been associated with improved conception rates of lactating ruminants. Similarly, progesterone concentration prior to AI has been associated with greater fertility. In a field study involving 426 lactating dairy cows, blood was sampled at 58 days postpartum for multiparous cows and 72 days for primiparous cows and then analyzed for

progesterone. Cows were bred approximately 3 days later in a synchronized estrus scheme. Conception rate increased 1.44% for every 1 ng/ml increase in plasma progesterone ( $r^2 = 0.11$ , Staples et al., 1997). The recovery of embryos 7 days after estrus increased as plasma progesterone concentration increased just prior to AI (Britt et al., 1996). In either association, dietary fat, which stimulates ovarian cyclicity or corpus luteum function, would contribute to increased fertility. Increased progesterone suggests that luteal function is enhanced by dietary fat. Dynamics of maternal progesterone secretion also appear important for conceptus development and secretion of interferon- $\tau$ , which is secreted by the embryo for gestation recognition by the mother.

It has been suggested that improved conception rate could be a result of increased concentrations in plasma cholesterol (Spicer et al., 1993), although this hypothesis was not supported by our results. In fact, cows fed formaldehyde-treated flaxseed had lower plasma cholesterol concentration and better conception rates than those fed Megalac<sup>®</sup> (Church and Dwight Co., Inc., Princeton, NJ) (Petit et al., 2001). Other studies have reported no relationship between cholesterol concentrations in blood and reproductive measures (Ferguson et al., 1990).

The fatty acid profile of the dietary fat may influence the propensity of animals to increase plasma progesterone. Mature ewes were infused intravenously with saline, soybean oil, or olive oil for 5 hours on days 9 through 13 of an estrous cycle (Burke et al., 1996). Serum cholesterol was increased by fat infusates, and olive oil was more effective than soybean oil (127, 141, and 153 mg/dl of serum cholesterol for saline, soybean oil, and olive oil, respectively). However, soybean oil infusion resulted in greater progesterone response than did infusion of olive oil at 2.5 hours postinfusion. Therefore, the greatest concentration of serum

cholesterol did not coincide with the greatest concentration of serum progesterone.

### Fatty Acids and Prostaglandin Secretion

It is known that there is a negative relationship between concentration of  $\text{PGF}_{2\alpha}$  and that of progesterone. For example, at calving,  $\text{PGF}_{2\alpha}$  concentration increases while that of progesterone decreases. Similarly, during gestation,  $\text{PGF}_{2\alpha}$  concentration decreases and that of progesterone increases. Progesterone is secreted by the corpus luteum and synthesized by steroids. Therefore, an increase in  $\text{PGF}_{2\alpha}$  concentration is paralleled with a decrease in progesterone concentration and vice versa. In theory, it could thus be possible to modulate concentrations of  $\text{PGF}_{2\alpha}$  and progesterone by different feeding strategies! In fact, in the experiment we carried out in the UK, we observed a tendency ( $P = 0.09$ ) for greater progesterone concentration in the blood of cows fed formaldehyde-treated flax compared to those fed Megalac<sup>®</sup> (Petit et al., 2002). This may partly explain the greater gestation rate observed for cows fed formaldehyde-treated flax (87.5%) compared to those fed Megalac<sup>®</sup> (50.0%) in a companion study (Petit et al., 2001).

Better conception rate for cows fed formaldehyde-treated flaxseed compared to those fed Megalac<sup>®</sup> could result from different PG synthesis. In fact, linolenic acid in flaxseed uses the eicosapentaenoic acid metabolic pathway, while fatty acids in Megalac<sup>®</sup> partly uses the arachidonic acid pathway, (Cunnane, 1995) and it is known that eicosapentaenoic acid inhibits PG synthesis (Spicer et al., 1993). Therefore, ingestion of linolenic acid contained in flaxseed could potentially inhibit  $\text{PGF}_{2\alpha}$  synthesis (Cunnane, 1995). Thatcher et al. (1997) has shown that  $\text{PGF}_{2\alpha}$  secretion is decreased in dairy cows fed fish meal, which is a source of omega 3 fatty acids. Moreover, feeding diets containing 2.6, 5.2, and 7.8% Menhaden fish meal to lactating dairy cows reduced uterine secretion of  $\text{PGF}_{2\alpha}$  (Thatcher et al., 2001a). In fact, fish meal,

which would lead to eicosapentaenoic acid and docosahexaenoic acid formation, has been shown to increase gestation rate of dairy cows and to alter corpus luteum regression as shown by greater plasma concentrations of progesterone (Burke et al., 1997). This would agree with the tendency observed in one of our experiments (Petit et al., 2002) for greater milk progesterone concentration, expressed as the area under the curve, for cows fed formaldehyde-treated flaxseed compared to those fed Megalac<sup>®</sup>. However, it is not known if the greater conception rate observed for cows fed formaldehyde-treated flaxseed in the experiment of Petit et al. (2001) was a result of a decrease in embryo mortality or better fertilization of the ova as pregnancy was confirmed only once at day 45 post AI. More research is required to determine the reasons for better conception rate for cows fed a source rich in omega-3 fatty acids. The potential to improve reproduction of dairy cows through dietary manipulation is an exciting concept and needs to be further addressed.

Dietary PUFA can decrease  $\text{PGF}_{2\alpha}$  synthesis by different actions, which include decreasing the availability of the precursor arachidonic acid, increasing the concentration of fatty acids that compete with arachidonic acid for series 2 PG, and inhibiting PG synthase. Reduced availability of arachidonic acid in the uterine phospholipid membranes for conversion to series 2 PG can occur through a reduction in the synthesis of arachidonic acid or through displacement of existent arachidonic acid from the phospholipid membranes by other fatty acids. This can be achieved through dietary supplementation with fish oil (rich in EPA and DHA) or linseed oil as they are major inhibitors of desaturation and elongation in liver cells, leading to arachidonic acid formation (Bezard et al., 1994). Moreover, as there is a preferential processing of n-3 fatty acids by  $\Delta 6$  desaturase at the expense of desaturation of n-6 fatty acids (Sprecher, 1981), feeding n-3 fatty acids would lead to a reduction in arachidonic acid formation. In summary, inhibition of PG secretion can be

achieved through: 1) reduced synthesis of arachidonic acid by  $\Delta 6$  and  $\Delta 5$  desaturase enzymes necessary for conversion of linoleic acid to arachidonic acid; 2) alteration in fatty acid profile (in favor of omega-3 fatty acids in membrane phospholipids which may or may not be precursors of other eicosanoids); 3) inhibition of synthesis and activity of cyclooxygenase enzymes responsible for the synthesis of  $\text{PGF}_{2\alpha}$ ; and 4) inhibition of gene expression involved in the synthesis of series 2 PG (Mattos et al., 2000).

### Maternal Recognition of Pregnancy

The dialogue between the conceptus and uterine endometrium leads to maintenance of the corpus luteum. The ability of embryonic interferon- $\tau$  to inhibit uterine secretion of  $\text{PGF}_{2\alpha}$  is critical to the establishment of pregnancy in cattle. Up to 40% of total embryonic losses are estimated to occur between day 8 and day 17 of pregnancy (Thatcher et al., 1994). This high proportion of losses is coincident with the period of conceptus inhibition of uterine  $\text{PGF}_{2\alpha}$  secretion, suggesting that some loss may be occurring because certain conceptuses are unable to inhibit secretion of  $\text{PGF}_{2\alpha}$ . Future strategies to improve embryo survival during this critical period will be based on a thorough understanding of the factors regulating “a better communication between the embryo and the mother at the embryo interface”.

The success of early pregnancy in the mated cow is dependant on the successful maternal recognition of pregnancy (Thatcher et al., 1995; Mann et al., 1999). To achieve this, the embryo must prevent the demise of the corpus luteum by the timely production of interferon tau, the embryonic signal which acts to inhibit the development of the maternal luteolytic mechanism. Interferon tau acts locally in the uterus to suppress the development of oxytocin receptors in the endometrium and thereby suppresses the secretion of luteolytic episodes of  $\text{PGF}_{2\alpha}$  generated by the binding of oxytocin to its receptors (Mann et al., 1999). It has been shown that the pattern and level of ovarian steroid hormones

in early pregnancy can influence both embryo development and survival and the timing and intensity of the mothers luteolytic drive. For example, low progesterone or high oestradiol concentrations during the luteal phase increase the strength of the luteolytic drive, while low post ovulatory progesterone concentrations result in retarded embryo development (Mann et al., 1999). Moreover, low estradiol concentrations may prevent premature regression of the corpus luteum and prevent early embryonic death (Staples et al., 1998).

Oxytocin induces release of  $\text{PGF}_{2\alpha}$  (Tysseling et al., 1998). Increased PG synthesis induced by oxytocin during days 5 to 8 of pregnancy reduced pregnancy rates of beef cows at 30 days after AI from 80 to 30% (Lemaster et al., 1999). Treating cows concomitantly with an inhibitor of prostanoid synthesis neutralized the effect of oxytocin and restored pregnancy rates to 80%. The implication is that increased  $\text{PGF}_{2\alpha}$  secretion during early pregnancy causes embryonic loss and supports the hypothesis that reducing  $\text{PGF}_{2\alpha}$  during this period reduces embryonic loss and improves pregnancy rates. Arachidonic acid is the rate limiting fatty acid for the synthesis of  $\text{PGF}_{2\alpha}$  via the action of  $\text{PGF}_{2\alpha}$  synthase. The same enzymes also are capable of processing other fatty acids, such as EPA, which is the precursor for the synthesis of prostanoids of the 3 series. Increased availability of EPA in membrane phospholipids could displace arachidonic acid, leading to increased synthesis of prostanoids of the 3 series at the expense of prostanoids of the 2 series, such as  $\text{PGF}_{2\alpha}$ . Prostanoids of the 3 series are less bioactive, and there appears to be no evidence for their role in ruminant luteolysis. Gamma-linolenic acid (GLA, C18:3n6) and EPA have been shown to reduce the synthesis in vitro of  $\text{PGF}_{2\alpha}$  and  $\text{PGE}_2$  (Graham et al., 1994).

Both EPA and interferon- $\tau$  inhibit secretion of  $\text{PGF}_{2\alpha}$  through different mechanisms. Interferon- $\tau$ , but not EPA, reduced levels of enzyme gene expression (cyclooxygenase-2) and thus modulates  $\text{PGF}_{2\alpha}$  production (Thatcher et al., 2001b). On the

other hand, EPA does not seem to affect enzyme gene expression but would be involved in competition of precursors for processing by the cyclooxygenase enzymes and regulation of enzyme activity. The implication of these findings is that supplementation with inhibitory fatty acids, such as EPA, during early pregnancy by dietary or parenteral means may further enhance the suppression of  $\text{PGF}_{2\alpha}$  secretion in concert with the action of embryonic interferon- $\tau$ . Because a significant proportion of bovine embryos are thought to be lost due to inadequate inhibition of uterine  $\text{PGF}_{2\alpha}$  secretion, further inhibition by exogenous means may result in increased embryo survival. This hypothesis is supported by the findings of Burke et al. (1997), in which feeding lactating dairy cows of low fertility a source of EPA and DHA in fish meal increased pregnancy rates from 31.9 to 41.3%.

### **Fatty Acids, Parturition, and Retained Placenta**

Parturition is a process that is accompanied by the massive release of PG. Alterations of fatty acids in the endometrium have been described in normal parturition and manipulations of fatty acid content used experimentally to delay onset of parturition. Fatty acids of the omega-3 family have been shown to affect uterine activity during parturition in rats and sheep and to delay the onset of parturition in humans (Olsen et al., 1992). Supplementing linolenic acid to a diet deficient in essential fatty acids resulted in an impairment of parturition rates (Leat and Horthrop, 1979). This also occurred when fish oil was given to rats as the major dietary essential fatty acid source, and an inhibition of uterine synthesis of  $\text{PGE}_2$  was detected (Leaver et al., 1986). In pre-term pregnant sheep, intravenous infusion of a 20% omega-3 fatty acid emulsion resulted in a delay in the onset of induced labour and delivery compared with a control group infused with an emulsion of soybean oil containing 7% omega-3 fatty acids.

Forages could also affect reproduction. Chassagne and Barnouin (1992) reported that cows fed grass silage had lower blood PG concentrations than those fed corn silage. Grass silage had a greater concentration of linolenic acid and lower concentration of linoleic acid than corn silage. As a result, the incidence of retained placenta in cows fed grass silage was higher than in cows fed corn silage. As linolenic acid is an inhibitor of PG secretion, a high linolenic to linoleic acid ratio (grass silage), therefore, could result in retained placenta. Kemp et al. (1998) reported that cows requiring more time to expulse their placenta also had lower PG metabolite blood concentrations at calving. However, in their experiment, the linolenic to linoleic acid ratio (C18:3:C18:2) had no effect on delivery time of placenta, probably because the difference in the linolenic to linoleic acid ratio between flaxseed and sunflower seed based diets was not large. This would suggest that fatty acid composition of forages and diets could have important effects on cow reproduction. So far, we have observed (Benchaar and Petit, unpublished results) that a grass silage based diet decreases the omega-6 to omega-3 fatty acid ratio in milk compared to a corn silage based diet; we have no data, however, on the effects of these two diets on reproduction parameters.

### **Fatty Acids and Reproduction Function**

In theory, feeding omega-3 fatty acids would delay the return to cyclicity after calving due to a decrease in the synthesis of series 2 PG, which could increase the number of days to first service. Synthesis of series 2 PG is required after calving for uterine involution, which leads to the return of normal cyclicity. On the other hand, feeding omega-3 fatty acids would improve maternal recognition and thus decrease embryo mortality. Taken altogether, this would strongly suggest that feeding omega-3 fatty acids would delay the return to cyclicity but lead to a better gestation rate when cows are bred. We are currently conducting an experiment to study this hypothesis and our preliminary results show that cows fed omega-3 fatty acids have no embryo

mortality compared to those fed micronized soybeans or calcium salts of palm oil (Petit and Twagiramungu, 2002).

Therefore, feeding omega-3 fatty acids should improve the overall reproductive function of cows as a result of better gestation rate, decreased embryo mortality, and decreased service per conception. However, we still need to do more research on this topic as there are almost no published data regarding the effects of specific fatty acids on the overall reproduction of dairy cows. There is a specific need to develop different feeding strategies according to the reproductive stage of cows; fatty acids required for better maternal recognition (omega-3) won't be necessary the same as those required for easier calving (omega-6). We should be balancing diets for specific fatty acids for optimum reproduction performance. The only problem is that polyunsaturated fatty acids (e.g. omega-3 and omega-6) are biohydrogenated by rumen microbes and these fatty acids must bypass the rumen to have any effect on reproduction. These fatty acids must therefore be protected against the attack of rumen microbes, but they must remain digestible in the intestine and this is even more important for free oils. Oils contained in fish meal (EPA and DHA) partially escape biohydrogenation in the rumen (Ashes et al., 1992).

### **Conclusions**

In a practical manner, we could summarize four possible strategies to improve reproduction of the cow:

- 1) Generate a larger corpus luteum: it is known that a larger corpus luteum will secrete more progesterone and this may have a positive effect on pregnancy recognition and consequently pregnancy rates. Feeding flaxseed has increased corpus luteum diameter and progesterone concentration in dairy cows (Petit et al., 2002).



2) Increase progesterone concentration: a greater progesterone concentration leads to a better maternal recognition of pregnancy (Staples et al., 1997). Feeding omega 3 fatty acids usually increases progesterone concentration.

3) Increase series 3 prostaglandin secretion: feeding fish meal (Burke et al., 1997) and flaxseed (Petit et al., 2002) inhibit  $\text{PGF}_{2\alpha}$  synthesis as both will lead to the synthesis of series 3 prostaglandins. Competition for the same key enzymes will lead to a lower synthesis of  $\text{PGF}_{2\alpha}$ .

4) Inhibition of cyclooxygenase activity: Cyclooxygenase is the enzyme leading to the synthesis of  $\text{PGF}_{2\alpha}$ . Eicosapentaenoic and docosahexanoic acids have been shown to inhibit cyclooxygenase activity. High concentrations of 20-carbon fatty acids (such as EPA, C20:5) other than arachidonic acid (C20:4n6), can compete with arachidonic acid for active sites of prostaglandin-endoperoxide synthase complex, therefore, reducing the conversion of arachidonic acid to the series 2 PG (Weber and Sellmayer, 1990).

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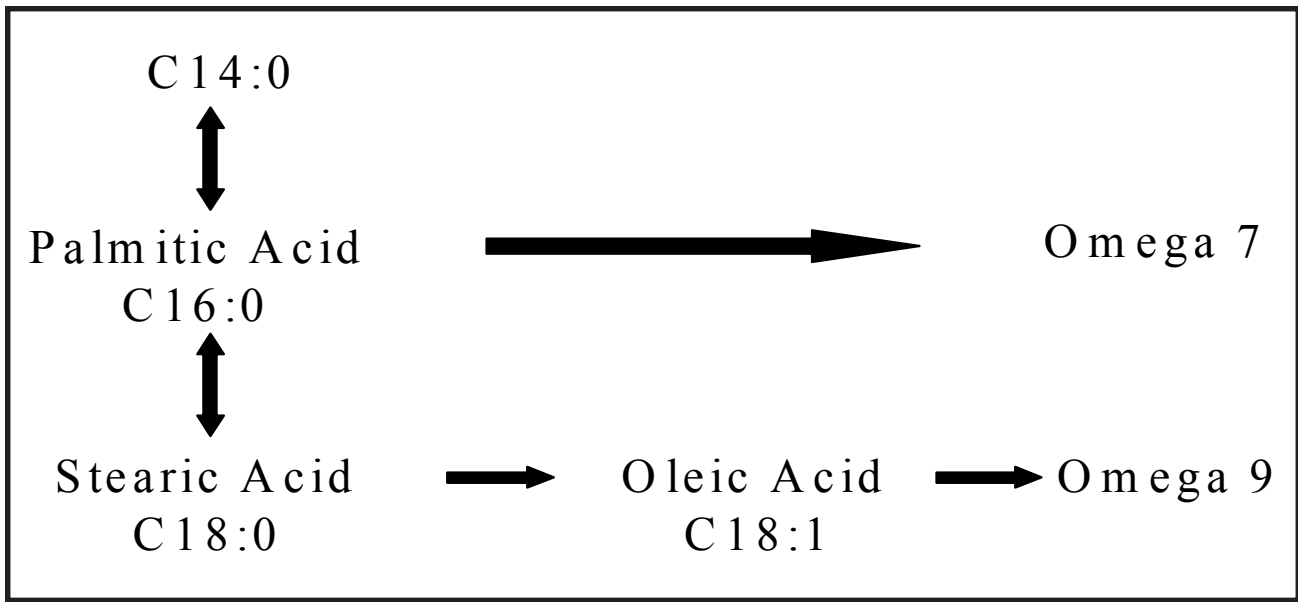
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**Table 1.** Comparison of major fatty acids in some edible oils (w/w% fatty acids)<sup>1</sup>.

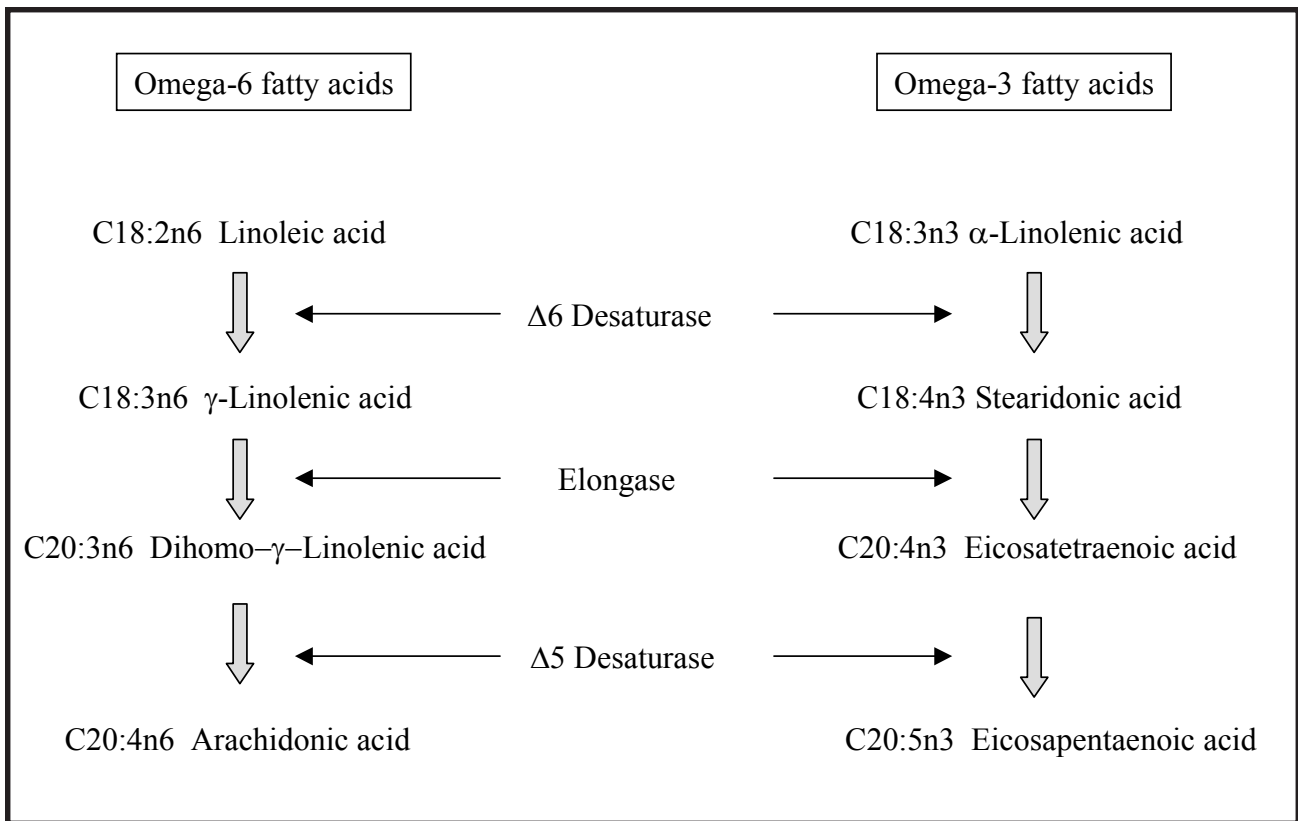
OIL	C18:0	C18:1	C18:2	C18:3
Peanut	2	47	32	0
Canola	2	64	19	8
Safflower	2	12	77	0
Cottonseed	25	21	50	0
Linseed (flax)	4	19	14	58
Corn	2	25	60	1
Tallow	15	41	8	1
Fishmeal (10 to 12 % fat)	2	25	4	45
Hi Linolenic Ryegrass	6	4	14	43
Olive	2	76	8	0
Palm	4	39	10	1
Sesame	2	42	45	0
Soybean	4	24	53	7
Sunflower	5	20	69	0
Megalac <sup>®2</sup>	3.5	32.3	7.8	0.3

<sup>1</sup>Adapted from Erasmus (1993).

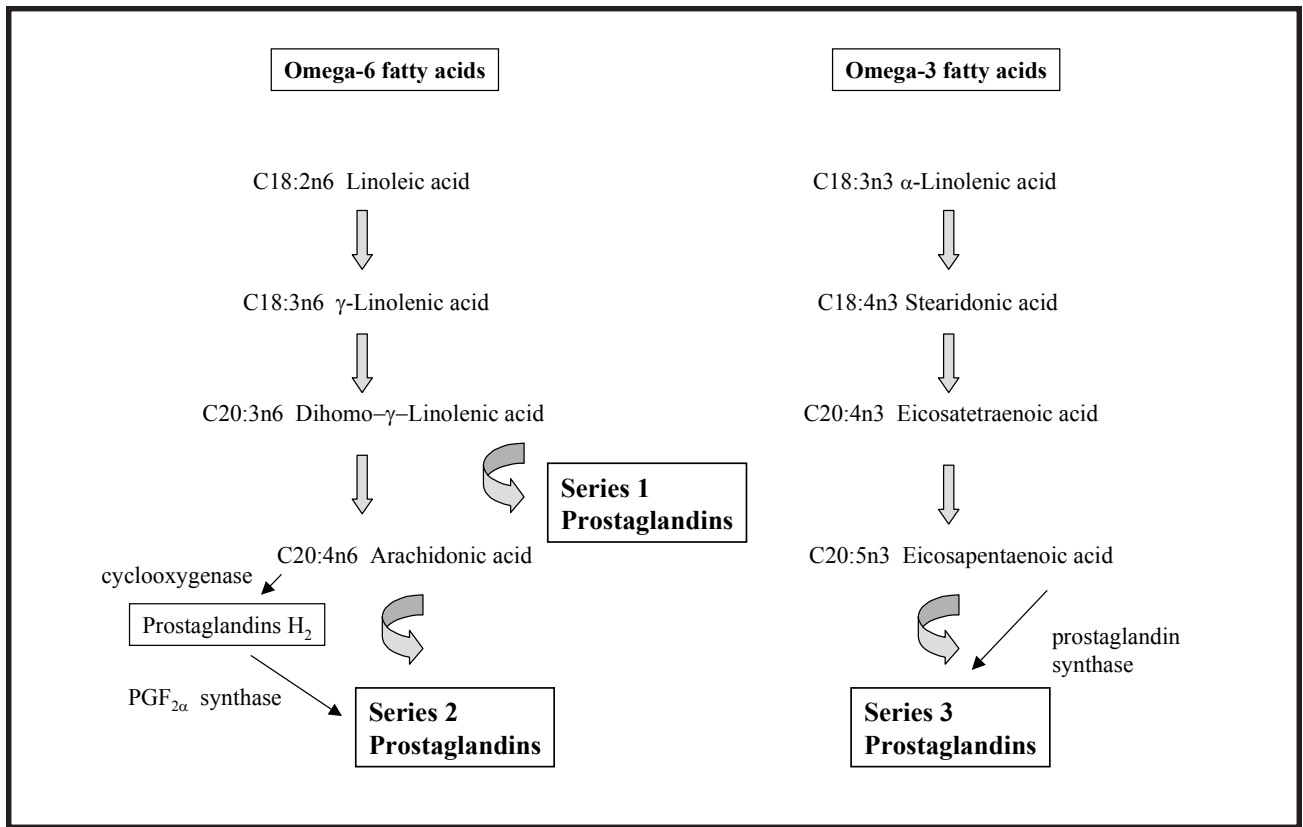
<sup>2</sup>Calcium salts of fatty acids. Church and Dwight Co., Inc., Princeton, NJ.



**Figure 1.** Schematic pathway of omega-7 and omega-9 fatty acid synthesis.



**Figure 2.** Schematic pathway of omega-6 and omega-3 fatty acid synthesis.



**Figure 3.** Metabolic pathway of series 1, 2, and 3 prostaglandins.



## Concentrations of Carbohydrates for Close-up Rations

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### Abstract

The major ingredient in dairy cow diets, regardless of physiological state, is carbohydrate. Altering the type and physical form of the carbohydrate fraction can vary the energetic value of diets for dairy cows. A close-up diet should not be formulated as an entity unto itself, but as a bridge between a low and high-energy diet. Therefore, it should retain some of the characteristics of both the far-off and lactation diets. The ultimate success of a transition cow nutrition and management program is a lactation characterized by high milk and component yields and an absence of ruminal, metabolic, mammary gland, and reproductive disorders. Therefore, close-up diets should encourage ruminal adaptation to subsequent lactation diets, prevent metabolic disorders, and minimize tissue mobilization prior to parturition. Rumen bacteria, protozoa, and fungi are sensitive to new diet ingredients and the amount of substrate available (DM intake) thus, adequate time should be allocated to close-up diet exposure prior to parturition. The total carbohydrate content of typical far-off, close-up, and lactation diets approximates 78, 74, and 68% of total dietary DM, respectively. The percentage of the carbohydrate fraction allocated to neutral detergent fiber (NDF) and non-fiber carbohydrate (NFC) in far-off, close-up, and lactation diets approximates 55, 45; 47, 53; and 40, 60%, respectively. The net effect of these changes is an increase in estimated net energy

but the actual energy value per unit will vary with DM intake.

### Introduction

The primary purpose of a close-up diet is to initiate ruminal changes beneficial to the cow after parturition when her nutritional demands dramatically increase. The far-off dry cow diet is generally high in NDF, moderate to low in NFC, and low in crude protein (CP) whereas, the lactation diet is relatively low in NDF, high in NFC, and high in CP. The close-up diet serves as a bridge between far-off and lactation diets thus, the primary changes in formulation should be the type of carbohydrate and, possibly, level of CP. The most appropriate changes to make are not clearly understood, but a recent study by Park et al. (2002b) suggests that the NFC content of the close-up diet should be above 32% of DM. Holcomb et al. (2001) fed prepartum diets containing high or low forage and reported no effect of forage level on postpartum performance, but their diets contained less than 32% NFC (25 and 30%, respectively). Hayirli et al. (2002) used data sets (49 diets) from 10 universities to evaluate animal and feed factors that affect feed intake during the prefresh transition period and demonstrated that dietary NDF accounted for 15.3% of the variation in DM intake of Holstein cows. Dietary NDF was negatively related, while NFC was positively related, to DM intake. Their (Hayirli et al., 2002) diets ranged in NDF content

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from 28.0 to 62.2% and NFC content from 10.5 to 46.2% of DM. We would expect that DM digestibility would increase as we replace NDF with NFC up to a point and then decrease when the rumen environment is compromised. Confounded with the issue of the most appropriate dietary NDF to NFC ratio is the digestibility of the NDF fraction. Varga (2002) suggests that highly fermentable non-forage fiber sources offer a means of stimulating intake in the prefresh cow by increasing rate of passage through the rumen. Ferdinand et al. (2002) improved DM intake by replacing a portion of the alfalfa hay, corn silage, and corn grain with either wet corn gluten feed or a soy hull-corn steep liquor blend in diets fed to early lactation cows. The obvious advantage of highly fermentable non-forage fiber sources is that they increase nutrient delivery to the cow without increasing the risk of acidosis.

The basic question left unanswered in the literature is how rumen microbes react to dietary changes dictated by the two-tier dry cow feeding program. This presentation will discuss ruminal adaptation to changes in diet and DM intake during the transition from far-off to close-up dry through early lactation and some factors to consider regarding the carbohydrate fractions when formulating close-up diets.

### **Composition of far-off, close-up, and lactation diets**

Formulations that have been successful in our program for far-off, close-up, and lactation diets are shown in Table 1 and the chemical composition in Table 2. These diets were offered as a total mixed ration (**TMR**) and fed for ad libitum intake. The major component changes between the far-off and close-up diets were the introduction of alfalfa hay, a decrease in prairie hay and corn grain, and an increase in corn silage and soybean meal.

These changes increased CP, NFC, and estimated  $NE_L$  and decreased NDF as a

percentage of total diet DM. Table 3 provides a different perspective of these diets. Total carbohydrates decrease as we transition from the far-off to lactation diet and a major shift in carbohydrate fractions occurs, NDF decreases, and NFC increases. The amount of corn grain was reduced in the close-up compared to the far-off diet because the amount of corn silage increased. A comparison of TMR particle size using the Penn State Particle Separator (Lammers et al., 1996) is shown in Table 4. The particle size is slightly less in the close-up compared to the far-off diet; the major change in particle size occurs in the lactation diet.

### **Intake and digestive characteristics of far-off, close-up, and lactation diets**

Dry matter intake (Table 5) averaged 2.3% of BW during the far-off and early close-up periods, then decreased to 1.97% of BW during the week before calving when the above diets were fed ad libitum. Actual intakes ranged from 15 to 20% higher than predicted by the dairy NRC (2001) for dry cows and cows during early lactation but were within 1% of actual at 90 days in milk. Dry matter intake increased slightly (31 to 32 lb/day) when cows were switched from the far-off to the close-up diet, but rumen fill decreased because solids passage rate increased (Tables 5, 6, and 7). These events were predictable because the close-up diet was formulated to improve digestibility. The digestibility characteristics of the diets during the transition period and through 90 days in milk are in Table 8. Dry matter, organic matter, CP, and NDF digestion increased as DM intake decreased during the close-up period. Digestibility of these major dietary components was less on day 20 of lactation than on day 6, likely due to the rapid increase in DM intake, and then gradually increased through day 90 of lactation. Solids and liquid passage rates were relatively constant between day 20 and 48, then gradually declined through day 90 of lactation. These results support the concept that the close-up diet prepared the ruminal microbes for the

lactation diet but not for the substantial increase in DM intake. This concept is further supported by our inability to detect lactic acid in ruminal samples throughout the study. The concentration of total ruminal volatile fatty acids (Table 9) gradually increased from day 51 prepartum (far-off diet) through day 34 postpartum, peaked on day 48 postpartum, and then gradually decreased through day 90 of lactation. Concentrations of acetate, propionate, and butyrate mimicked the pattern of total volatile fatty acids, while the concentrations of valerate, isobutyrate, and isovalerate tended to peak by day 20 postpartum (Table 10). These results are consistent with the pattern of intake, DM fill, organic matter fill, and solids passage rate and retention time.

### General comments about close-up diets

Properly formulated close-up diets can reduce the incidence of postpartum metabolic problems and prepare the rumen for lactation diets; however, it is not a magic formula that can cure problems created by overly fat cows and low quality or improperly formulated lactation diets. In our view, cows should enter the close-up pen at a body condition score of 2.75 to 3.0 and gain 0.25 points prior to calving. To accomplish this, cows should enter the close-up pen approximately 28 days prior to expected calving date to insure that they are exposed to the close-up diet for a minimum of 14, but hopefully 21 days. Park et al. (2000) reported that body condition score at the beginning of the close-up period (day 31 prepartum) was negatively related to prepartum and postpartum DM intake (first 90 days) and milk yield during the first 90 days of lactation but not significantly related to complete lactation milk yield. These data support the concept that fatter cows eat less than thinner cows and suggest that energy gained from fat mobilization during early lactation does not offset the decrease in DM intake experienced by fatter cows in support of milk production. Hayirli et al. (2002) also reported a negative relationship between body condition score and prepartum DM intake.

The 2001 Dairy NRC recommends that close-up diets contain 0.73 Mcal  $NE_L$ /lb of dietary DM. The close-up diet discussed in this presentation contained 0.71 Mcal  $NE_L$ /lb of DM based on the summation of values from individual feedstuffs and 0.74  $NE_L$ /lb dry matter based on NRC 2001. A significant portion of the  $NE_L$  in our close-up diet is due to the CP content (15.5% of DM), so the recommended NEL value can be achieved without a substantial increase in the rapidly fermentable carbohydrate fraction.

### Summary

The primary purpose of a close-up diet is to initiate ruminal changes beneficial to the cow after parturition when her nutritional demands dramatically increase. Rumen bacteria, protozoa, and fungi are sensitive to new diet ingredients and the amount of substrate available thus, adequate time should be allocated to close-up diet exposure prior to parturition. Altering the type and physical form of the carbohydrate fraction can vary the energetic value of diets. Close-up diets containing 35% NFC and 14.5% CP should stimulate fermentation sufficiently to prepare the rumen for the lactation diet. Properly formulated close-up diets can reduce but cannot completely solve the problem of over fat cows and low quality feedstuffs.

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**Table 1.** Diet ingredients (% of DM; Adapted from Park et al., 2001).

Item	Diets		
	Far-off	Close-up	Lactation
Alfalfa hay	—	15.0	30.0
Prairie hay	48.4	20.0	—
Corn silage	19.8	30.0	15.0
Corn grain	22.4	18.7	32.0
Whole cottonseed	—	—	9.3
Fishmeal	—	—	1.3
Expeller soybean meal	—	9.4	3.3
48% soybean meal	8.4	4.4	4.4
Wet corn gluten feed	—	—	—
Molasses	—	—	1.0
Limestone	0.06	0.60	1.36
Dicalcium phosphate	0.40	0.74	0.88
Sodium bicarbonate	—	—	0.75
Trace mineral salt <sup>1</sup>	0.34	0.50	0.32
Magnesium oxide	—	0.50	0.21
Vitamin A,D,E <sup>2</sup>	0.11	0.12	0.13
Sodium selenite premix <sup>3</sup>	0.02	0.04	0.01

<sup>1</sup>Composition: not less than 95.5% NaCl, 0.24% Mn, 0.24% Fe, 0.05% Mg, 0.032% Cu, 0.032% Zn, 0.007% I, and 0.004% Co.

<sup>2</sup>Contributed 4912 IU vitamin A, 2358 IU vitamin D, and 24 IU vitamin E per kg diet DM.

<sup>3</sup>Contributed 0.06 mg Se per kg diet DM.

**Table 2.** Chemical characteristics of diets (adapted from Park et al., 2001).

Item	Diets		
	Far-off	Close-up	Lactation
DM, %	74.5	70.3	75.0
CP, %	11.5	15.6	18.4
Soluble protein, % of CP	25.2	25.2	31.3
RDP, % of DM <sup>1</sup>	7.3	10.3	11.7
RUP, % of DM <sup>1</sup>	4.2	5.3	6.7
ADF, %	25.2	22.0	18.2
NDF, %	42.9	34.4	27.0
Non-fiber carbohydrate, % <sup>2</sup>	35.2	39.1	40.4
NE <sub>L</sub> , Mcal/lb <sup>3</sup>	0.70	0.74	0.73
Ether extract, %	3.8	3.5	5.6
Ash, %	6.7	7.4	8.4
TDN, %	67.0	69.1	72.3
Calcium, %	0.5	0.8	1.5
Phosphorus, %	0.4	0.5	0.7
Magnesium, %	0.2	0.4	0.3
Potassium, %	1.2	1.5	1.5
Sodium, %	0.1	0.2	0.3
Sulfur, %	0.1	0.2	0.2

<sup>1</sup>Based on feed analysis from Dairy Herd Improvement Forage Testing Laboratory (Ithaca, NY).

<sup>2</sup>Calculated based on DHI formula represented by 100- [(crude protein + (NDF – NDICP) + ether extract + ash)].

<sup>3</sup>Calculated based on NRC, 2001. Estimates of NE<sub>L</sub> values from summation of individual ingredients (0.66, 0.71, and 0.77 for the far-off, close-up, and lactation diets, respectively).

**Table 3.** Carbohydrate fractions in dry and lactating cow diets (adapted from Park et al., 2001).

Item	Far-off	Close-up	Lactation
CP, % of DM	11.5	15.6	18.4
Ether extract, % of DM	3.8	3.5	5.6
Ash, % of DM	6.7	7.4	8.4
Carbohydrates, % of DM	78	73.5	67.6
NDF, % of CHO <sup>1</sup>	55	47	40
NFC, % of CHO <sup>1</sup>	45	53	60

<sup>1</sup>CHO = carbohydrate.

**Table 4.** Particle size characteristics of diets (adapted from Park et al., 2001).

Item	Diets		
	Far-off	Close-up	Lactation
Total mixed ration particle size, % <sup>1</sup>			
> 19.0 mm	34.1 ± 12.6	30.2 ± 11.8	11.2 ± 4.6
8.0 to 19.00 mm	20.1 ± 01.3	19.1 ± 05.2	24.9 ± 3.3
< 8.0 mm	45.8 ± 12.9	50.7 ± 08.0	63.9 ± 3.4

<sup>1</sup>Particle size determined by the Penn State Particle Separator (Lammers et al., 1996), as-fed basis; mean ± SD.

**Table 5.** Performance characteristics (adapted from Park et al., 2001)<sup>1</sup>.

Day	Diet	F:C	NRC		BW (lb)	DMI (% of BW)	BCS	EB (Mcal/day)
			DMI (lb/day)	DMI (lb/day)				
-51	Far-off	70 : 30	31.1	25.4	1365	2.29	2.75	8.5
-23	Close-up	65 : 35	32.2 <sup>a</sup>	27.1	1417	2.29 <sup>d</sup>	2.75 <sup>a</sup>	10.3
-9	Close-up	65 : 35	28.2 <sup>ab</sup>	25.4 <sup>a</sup>	1465 <sup>d</sup>	1.97 <sup>ad</sup>	2.94 <sup>ab</sup>	7.3
6	Lactation	45 : 55	36.2 <sup>b</sup>	49.4 <sup>a</sup>	1312 <sup>d</sup>	2.70 <sup>a</sup>	2.50 <sup>b</sup>	(2.0)
20	Lactation	45 : 55	45.2	36.4	1293	3.55	2.56	8.3
34	Lactation	45 : 55	53.6	39.5	1263	4.29	2.38	14.9
48	Lactation	45 : 55	56.2	41.5	1284	4.43	2.25	17.0
62	Lactation	45 : 55	56.9	42.6	1249	4.58	2.19	19.1
76	Lactation	45 : 55	57.8	51.8	1179	4.93	2.13 <sup>c</sup>	11.3
90	Lactation	45 : 55	58.2	57.1	1211	4.81	2.38 <sup>c</sup>	7.4
SEM			2.0	8.6	144	0.20	0.18	5.9

<sup>abc</sup>Like superscripts within column differ by ( $P < 0.05$ ).

<sup>d</sup>Like superscripts within column tend to differ by ( $P < 0.10$ ).

<sup>1</sup>FC=Forage to concentrate ratio, DMI = dry matter intake, NRC = National Research Council (2001), BW = body weight, BCS=body condition score, EB = energy balance, and SEM = standard error of mean.

**Table 6.** Rumen capacity and fill (adapted from Park et al., 2001).<sup>1</sup>

Day	Diet	F:C	Total fill (lb/day)	DM fill (lb/day)	OM fill (lb/day)	Liquid fill (lb/day)	WHC (L)
-51	Far-off	70 : 30	132.5 <sup>c</sup>	13.6 <sup>c</sup>	11.7 <sup>c</sup>	118.9	124.6
-23	Close-up	65 : 35	117.3 <sup>c</sup>	10.7 <sup>ac</sup>	8.8 <sup>ac</sup>	106.5	139.6
-9	Close-up	65 : 35	111.0 <sup>a</sup>	13.4 <sup>ab</sup>	11.5 <sup>ab</sup>	97.6 <sup>a</sup>	143.1 <sup>c</sup>
6	Lactation	45 : 55	138.9 <sup>a</sup>	19.3 <sup>b</sup>	16.5 <sup>b</sup>	119.5 <sup>a</sup>	159.8 <sup>c</sup>
20	Lactation	45 : 55	134.7	19.4	16.8	115.3	148.7
34	Lactation	45 : 55	152.5	21.7	18.5	130.8	170.0
48	Lactation	45 : 55	146.4	21.9	19.0	124.5	164.3
62	Lactation	45 : 55	162.9	24.9	21.4	137.9	160.3
76	Lactation	45 : 55	162.1	25.0	21.2	137.1	169.7
90	Lactation	45 : 55	170.6	25.1	21.8	145.5	171.4
SEM			9.5	1.7	1.5	8.2	9.4

<sup>ab</sup>Like superscripts within column differ by ( $P < 0.05$ ).

<sup>c</sup>Tended to be different ( $P < 0.1$ ).

<sup>1</sup>FC = Forage to concentrate ratio, DM = dry matter, OM = organic matter, WHC = water holding capacity, and SEM = standard error of mean.

**Table 7.** Passage rate and retention time (adapted from Park et al., 2002a).<sup>1</sup>

Day	Diet	F:C	Solids (% / h)	Solid retention time (h)	Liquid (% / h)	Liquid retention time (h)
-51	Far-off	70 : 30	5.0 <sup>a</sup>	20.7	12.6	8.2
-23	Close-up	65 : 35	6.5 <sup>ab</sup>	17.4 <sup>a</sup>	12.0	8.3
-9	Close-up	65 : 35	4.5 <sup>bc</sup>	22.3 <sup>ab</sup>	12.2	8.2
6	Lactation	45 : 55	3.3 <sup>c</sup>	31.0 <sup>b</sup>	13.2	7.6
20	Lactation	45 : 55	3.8	27.2	14.4	7.0
34	Lactation	45 : 55	4.0	25.9	13.1	7.8
48	Lactation	45 : 55	4.4	23.4	14.1	7.2
62	Lactation	45 : 55	4.0	25.8	11.8	8.6
76	Lactation	45 : 55	3.8 <sup>d</sup>	27.0 <sup>c</sup>	12.7	8.3
90	Lactation	45 : 55	3.0 <sup>d</sup>	35.2 <sup>c</sup>	11.8	8.8
SEM			0.5	2.8	1.2	0.9

<sup>abcd</sup>Like superscripts within column differ by ( $P < 0.05$ ).

<sup>1</sup>FC = Forage to concentrate ratio and SEM = standard error of mean.

**Table 8.** Digestibility characteristics (adapted from Park et al., 2002a).<sup>1</sup>

Day	Diet	F:C	Percentage					
			DM	OM	CP	NDF	ADF	Starch
-51	Far-off	70 : 30	45.9 <sup>a</sup>	49.1 <sup>a</sup>	42.9 <sup>a</sup>	45.2	45.5 <sup>e</sup>	100.0
-23	Close-up	65 : 35	50.4 <sup>ab</sup>	54.2 <sup>ab</sup>	52.2 <sup>ab</sup>	49.5	50.5 <sup>ae</sup>	99.4
-9	Close-up	65 : 35	54.5 <sup>bc</sup>	58.0 <sup>b</sup>	65.8 <sup>b</sup>	48.0 <sup>a</sup>	44.5 <sup>ab</sup>	94.9 <sup>e</sup>
6	Lactation	45 : 55	58.3 <sup>c</sup>	60.1	62.4	62.1 <sup>a</sup>	63.0 <sup>b</sup>	89.2 <sup>e</sup>
20	Lactation	45 : 55	50.7	53.0	52.8	58.4	57.1	80.9
34	Lactation	45 : 55	55.3	58.1	59.0	52.2	40.7	85.2
48	Lactation	45 : 55	53.2	55.4	55.8	55.9	42.8	80.7
62	Lactation	45 : 55	52.1	54.5	56.0	58.5	43.1	76.0
76	Lactation	45 : 55	55.4 <sup>d</sup>	57.4 <sup>c</sup>	55.9 <sup>c</sup>	59.5	48.0 <sup>c</sup>	81.3
90	Lactation	45 : 55	64.1 <sup>d</sup>	66.1 <sup>c</sup>	66.2 <sup>c</sup>	59.4	56.4 <sup>c</sup>	83.0
SEM			1.4	1.3	1.6	1.8	1.7	2.4

<sup>abcd</sup>Like superscripts within column differ by ( $P < 0.05$ ).

<sup>e</sup>Tended to be different ( $P < 0.1$ ).

<sup>1</sup>FC = Forage to concentrate ratio, DM = dry matter, OM = organic matter, CP = crude protein, NDF = neutral detergent fiber, ADF = acid detergent fiber, and SEM = standard error of mean.

**Table 9.** Fermentation products and pH (adapted from Park et al., 2002b).<sup>1</sup>

Day	Diet	F:C	pH	A:P ratio	Concentration, mM			
					TVFA	peptides	FAA	ammonia
-51	Far-off	70 : 30	6.59	3.88 <sup>a</sup>	87.4	—	0.5	12.3
-23	Close-up	65 : 35	6.59	3.46 <sup>ab</sup>	94.1	0.2	1.0	9.6
-9	Close-up	65 : 35	6.65 <sup>a</sup>	4.28 <sup>bc</sup>	93.8 <sup>a</sup>	0.2 <sup>a</sup>	0.5 <sup>a</sup>	6.9 <sup>a</sup>
6	Lactation	45 : 55	6.28 <sup>a</sup>	3.52 <sup>c</sup>	109.6 <sup>a</sup>	2.0 <sup>a</sup>	2.6 <sup>a</sup>	12.3 <sup>a</sup>
20	Lactation	45 : 55	6.05	3.47	124.2	2.2	1.8	6.1
34	Lactation	45 : 55	6.29	3.84	123.7 <sup>b</sup>	2.4	1.2	7.9
48	Lactation	45 : 55	6.15	3.96	153.2 <sup>b</sup>	2.5	2.9	9.3
62	Lactation	45 : 55	6.14	3.26	131.0	1.1	1.8	7.1
76	Lactation	45 : 55	6.11	3.17	125.9 <sup>c</sup>	1.4 <sup>b</sup>	2.9 <sup>b</sup>	9.8
90	Lactation	45 : 55	6.20	3.18	110.2 <sup>c</sup>	1.2 <sup>b</sup>	1.6 <sup>b</sup>	9.5
SEM			0.09	0.16	5.9	0.3	0.6	1.9

<sup>abc</sup>Like superscripts within column differ by ( $P < 0.05$ ).

<sup>1</sup>FC = Forage to concentrate ratio, A:P = acetate:propionate, TVFA = total volatile fatty acids, FAA = free amino acids, and SEM = standard error of mean.



**Table 10.** Ruminal volatile fatty acids (adapted from Park et al., 2002a).<sup>1</sup>

Day	Diet	F:C	Concentration, mM					
			acetate	propionate	butyrate	valerate	isobutyrate	isovalerate
-51	Far-off	70 : 30	60.5	15.7	9.5	0.9 <sup>a</sup>	1.1	1.1 <sup>a</sup>
-23	Close-up	65 : 35	63.9	18.6	9.7	1.2 <sup>a</sup>	1.4	1.7 <sup>a</sup>
-9	Close-up	65 : 35	65.0	15.3 <sup>a</sup>	9.2 <sup>a</sup>	1.1 <sup>b</sup>	1.3 <sup>a</sup>	1.5 <sup>b</sup>
6	Lactation	45 : 55	73.1	21.5 <sup>a</sup>	12.5 <sup>a</sup>	1.7 <sup>b</sup>	1.6 <sup>a</sup>	1.9 <sup>b</sup>
20	Lactation	45 : 55	79.6	23.1	14.3	2.1	1.9	2.5
34	Lactation	45 : 55	80.1	20.8 <sup>b</sup>	13.5	1.7 <sup>c</sup>	1.7	2.1
48	Lactation	45 : 55	106.7	27.2	14.1	2.1	1.3	2.0
62	Lactation	45 : 55	84.1	26.0 <sup>b</sup>	15.3	2.1 <sup>c</sup>	1.5	2.2
76	Lactation	45 : 55	79.7 <sup>a</sup>	25.6 <sup>c</sup>	14.1 <sup>b</sup>	1.9 <sup>d</sup>	1.5 <sup>b</sup>	1.8 <sup>c</sup>
90	Lactation	45 : 55	69.1 <sup>a</sup>	21.9 <sup>c</sup>	12.3 <sup>b</sup>	1.6 <sup>d</sup>	1.8 <sup>b</sup>	2.1 <sup>c</sup>
SEM			3.9	1.7	0.6	0.1	0.1	0.1

<sup>abcd</sup>Like superscripts within column differ by ( $P < 0.05$ ).

<sup>1</sup>FC = Forage to concentrate ratio and SEM = standard error of mean.

## Soluble Carbohydrates for Lactating Dairy Cows

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### Abstract

A major challenge in ration formulation is to provide an adequate amount of soluble carbohydrates in the diet to maintain high production, while at the same time assuring prevention of ruminal acidosis. The soluble carbohydrates include organic acids, sugars, starch, and soluble fiber, such as pectic substances, and are defined as non NDF carbohydrates (NFC). It has been suggested to restrict NFC to 32 to 38 % of ration dry matter (DM) when carbohydrates are derived primarily from sugar or starch or 38 to 42% when other soluble carbohydrates make up the ration. The contribution of various NFC is critical as they differ in their rate and extent of fermentation in the rumen and can have variable effects on rumen pH. Sugar and starch may ferment to lactic acid, while pectic substances ferment rapidly to acetate and lactate, but their fermentation is depressed at lower pH. To minimize the risk of ruminal acidosis and maximize the provision of rapidly fermentable NFC in the ration of dairy cows, a balance of these carbohydrate sources is recommended.

### Introduction

Carbohydrate nutrition of the high producing dairy cow is of extreme importance for the achievement of optimum performance but is perhaps the most difficult nutrient to adequately balance in the ration. Part of the

problem is that there is an inadequate description of the carbohydrate content of various feed ingredients and partly because of limitations for utilization of certain materials residing at the farm. In order for high producing dairy cattle to meet their high energy demands, diets must consist of large quantities of concentrates and high quality forages containing relatively low amounts of fiber. However, to maintain normal rumen function and milk fat percentage, a large portion of the fiber needs to come from forage. Sugars, starches, and other reserve carbohydrates, such as galactans and pectin, make up the highly digestible NFC and are the primary dietary sources of energy for the high producing dairy cow. Ruminant diets vary in NFC, and this can have a major impact on the end products of fermentation in the ruminal environment. These soluble carbohydrates are highly digestible and ruminal fermentation varies greatly with type and processing method. Understanding how these fermentable carbohydrate sources fit in the ration and how they differ in the nutrients they supply to the animal, will offer a better sense of how we should consider them in ration formulation.

### NFC

Dietary NFC are generally categorized into organic acids, sugars (mono- and oligosaccharides), starch, and neutral detergent-soluble fiber (Hall and Van Horn, 2001; Figure 1). Organic acids from fermented feeds may be

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utilized by the animal but do not support appreciable microbial growth. Sugars ferment quite rapidly in the rumen environment and also can be digested by mammalian enzymes. Starch may be digested both by ruminal microorganisms and by digestive enzymes post ruminally. However, there can be great variation in rate of ruminal fermentation or digestion depending on the processing, storage method, or plant source of the starch. Neutral detergent-soluble fiber includes pectic substances, beta-glucans, fructans, and other non-starch polysaccharides not included in neutral detergent fiber (**NDF**). Generally, most soluble fiber sources tend to ferment very rapidly (20 to 40%/h), while others, such as soyhulls, are digested at a much slower rate. Soluble fiber cannot be digested by mammalian enzymes and must be fermented by ruminal microbes to be utilized by the animal. Ruminal fermentation of sugar, starch, and fructan may yield lactic acid and tend to yield more propionate than acetate. Pectins, which usually predominate in soluble fiber, tend to yield more acetate. Other than organic acids, the various soluble carbohydrates have been considered to give similar yields of microbial protein when pH is relatively neutral and fermentation rates are similar (Hall and Van Horn, 2001). Table 1 provides a list of some feedstuffs, their particular NFC make-up, and the potential end products formed during ruminal fermentation.

#### *Organic acids*

Plant organic acids include citrate, malate, quinate, succinate, fumarate, oxalate, shikimate, *trans*-aconitate, and malonate among others. The range of organic acids present in forage has made complete analysis for them difficult (Hall, 2000). Total levels in plant DM reported in cool season grasses are 1.3 to 4.5%, 5.8 to 9.8% for alfalfa, 2.8 to 3.8% in red clover, and 3.0 to 3.5% in white clover (Dijkshoorn, 1973). Alfalfa varieties may contain 2.9 to 7.5% malate (Callaway et al., 1997), while citrus peel contains 3 to 4% of DM as organic acids

(Vandercook, 1977). The volatile fatty acids (**VFA**) are the fermentation acids in feedstuffs, such as silages, and contribute to the energy that the cow receives from the fermented feed but provide little or no fermentable carbohydrate for the rumen (Hall, 2000). However, as it relates to the animal, VFA can contribute as much as 150 to 300 g of absorbed amino acids per day, which is quite significant (Fox et al., 1992).

#### *Sugars*

Glucose, fructose, and sucrose are the predominant low molecular weight carbohydrates in forages. The water-soluble cell contents are reported to account for 1 to 3% of forage DM for the simple sugars and 2 to 8% for sucrose in temperate forages (Smith, 1973). Some of the by-products from the food industry, such as bakery and cereal, can vary widely in sugar content. Candy, donuts, and sugar-based cereals will be higher in sugar than bread products or bran type cereals. Analysis of 17 samples of citrus pulp showed an average sugar content of 25.9%, with a range of 19 to 31%. Beet pulp is lower in sugar (12%) than citrus pulp (Aldrich, 2001). Beet molasses is generally 40% sugar on an as-is basis, while whey is approximately 70% sugar (lactose). Almond hulls contain 19 to 34% soluble sugars (Aguilar et al., 1984), varying by variety. Other feedstuffs and their sugar composition are provided in Table 2.

Simple sugars and oligosaccharides are the most rapidly fermented of carbohydrate sources by ruminal microorganisms. The fermentation of sugars is similar to that of starch in that both can ferment to lactic acid. End products of sucrose fermentation are dependent on other carbohydrate sources in the diet that may contribute to variability in pH. Table 3 provides the proportion of carbohydrates converted to substrate based on all forage versus 50% forage rations. In the study conducted by Strobel and Russell (1986), fermentation of sucrose by rumen microbes resulted in similar

concentrations of microbial protein, acetate, and propionate as compared to starch, but more butyrate and lactate at pH 6.7. However, at a more acidic pH (5.5), fermentation of sucrose produced more lactate than did starch, and microbial protein yield from sucrose was reduced by 34%.

An *in vitro* study with mixed ruminal microbes examined the effects of different levels of sucrose (65, 130, and 195 mg) fermented with bermudagrass NDF (130 mg) on microbial products (Hall, 2002). The maximal yield of microbial protein increased linearly with increasing sucrose, and the efficiency of production decreased linearly from 0.32 to 0.23 (mg of microbial CP / mg of sucrose). Dextran yield peaked at four hours of fermentation and gradually declined (Table 4). Reports of inefficiency of CP utilization in diets containing molasses compared to those containing corn (Bell et al., 1953) may be related to sucrose-utilizing microbes storing dextran to use for maintenance rather than growth. Concentrations of all the VFA and lactate increased linearly ( $P < 0.01$ ) with increasing sucrose (Hall, 2002) (Table 5). It appears that yields of microbial products (dextran, microbial protein, and types of organic acids) usable by the animal to meet nutrient requirements may change with changing level of sucrose. In studies conducted by Khalili and Huhtanen (1991), and Huhtanen and Khalili (1991) sucrose and molasses fed at high levels decreased ruminal fiber digestion. Martin et al. (1981) demonstrated, however, that increasing the amount of protein in molasses-supplemented rations can significantly improve the digestibility of fiber. The relationship between fiber digestibility and protein supplementation on molasses diets may be related to a competition for ammonia nitrogen between fiber and NFC fermenting bacteria (Jones et al., 1998). Adequate nitrogen must be supplemented to the rumen to avoid starving fiber digesters, especially if rapidly growing NFC bacteria are scavenging available nitrogen.

Few studies have examined the addition of sucrose to lactating cow diets, and field observations have been mixed. Corn silage can vary from 1 to 5% sugar on a DM basis. Hay crops are generally higher than ensiled haylages. Therefore ensiled feeds, depending on their quality and fermentation process, can vary in sugar content. The response to sugar supplementation could vary based on the amount of total sugar needed in the diet and the amount already present in forages. Optimum sugar levels have not been well researched and certainly will depend as previously mentioned on the amount of readily available protein in the diet. Animal responses to added sugar may be highest when the basal diet contains 4% sugar or less. However, the challenge is to have sugar analyses available on all ingredients prior to sugar addition. Two studies in which sucrose was substituted for starch in lactating dairy cow rations suggest that sucrose increases butterfat yield, but other results are varied. In diets where sucrose was substituted for corn starch (0 to 7.5% of dietary DM, diet NFC ~ 43% of DM; Broderick et al., 2000), there were increases in DM intake, milk fat content, and fat yield. Fat-corrected milk production tended to increase (Table 6). In terms of feed efficiency, milk / DM intake decreased from 1.60 to 1.52, and the conversion of ration nitrogen to milk protein N declined linearly with increasing substitution of sucrose for starch (from ~0.31 to ~0.29). A continuous culture study was conducted to compliment this lactating cow study (Varga et al., 2001). Replacing corn starch with sugar in the diet altered fermentation of ruminal end products, such that molar proportions of butyrate were increased and may explain the increase in milk fat percentage in the study of Broderick et al. (2000). In addition, at the highest level of sugar inclusion, fiber digestibility was enhanced six percentage units. Increased bacterial nitrogen flow as a percentage of total nitrogen flowing as sugar replaced corn on the diet, may also explain the increase in milk yield and trend for the increase in milk protein observed in the companion lactating cow trial.

In a study conducted by Nombekela and Murphy (1995), sucrose was substituted for corn meal at 1.5% of ration DM. Intake, milk yield, and fat-corrected milk yield did not change, but milk fat yield increased from 2.12 to 2.14 lb/day, and milk protein decreased from 3.51 to 3.28%. The lower milk protein percentage may have been the result of reduced microbial protein production due to a decrease in ruminal pH and the negative effects on fiber digestibility. Therefore, for sugar to be effective in lactating cow rations, basal sugar levels need to be known, adequate rumen degradable protein needs to be fed, and efficiency of microbial CP is likely dependent on level of sucrose and other soluble carbohydrates in the diet.

Varga and Whitsel (1991) demonstrated that greater than 95% of the variation in rate and extent of fiber degradation could be explained by the proportion of sugars in the diet. In addition, the carbohydrate level at which the diet is formulated is important. For example, if a ration is formulated to contain 25% NDF on a DM basis, its relationship to NFC will be different than when formulated to contain 30% NDF. This in part is related to the effect of the fermentability of the carbohydrate source and its effects on ruminal pH and ultimately fiber digestion in the rumen.

### *Starch*

Fermentation of starch by rumen microbes has a variety of similarities to that of sugars. Starch may ferment to lactate (Strobel and Russell, 1986) and tends to produce a lower acetate to propionate ratio than cell wall carbohydrates. Although starch-fermenting bacteria are more tolerant of acidic conditions than are fiber digesters, growth of starch-digesting microbes declines as pH declines (Therion et al., 1997). Strobel and Russell (1986) demonstrated that the yield of microbial protein decreased by 35% when starch was fermented by mixed rumen microbes at pH 5.8 versus 6.7.

The rate and extent of starch digestion is affected by a variety of factors. Particle size, grain type, steam flaking, and preservation method (dry or ensiled) all affect the availability of starch. In feeds such as corn, the smooth covering of the seed offers the first barrier to digestion, and the protein matrix that surrounds the starch granules the second (Kotarski et al., 1992). For whole grain, approximately 30% may pass undigested into the manure in cattle (Orskov, 1986). But as particle size in whole corn decreases, ruminal starch disappearance generally increases (Gaylean et al., 1981). Processing methods which disrupt the protein matrix around the starch granules have been shown to increase grain digestibility (Lykos and Varga, 1995). Subjecting starch to heat and moisture gelatinizes it, destroying the crystalline structure of starch granules and increasing digestibility in the rumen and total tract (Lykos et al., 1997). It has been suggested that overall metabolizable energy yield to the cow is best when starch is fermented in the rumen, due to possible limitations on its digestion in the small intestine (Huntington, 1997). However, if digestion of starch in the small intestine were enhanced, such as with more post ruminal protein supply, it may be possible to improve the animal's capture of glucose from starch (Huntington, 1997).

In order to avoid acidosis and other metabolic problems, the maximum level of true sugars plus starches should not exceed 30% of the ration DM. This has resulted in the following guidelines: 32 to 38 % of DM as NFC when the dietary ingredients are high in sugar and starch, such as barley, corn, and corn silage; 38 to 42% of DM as NFC when the forage is all hay crop silage and contains by-products such as corn gluten feed and soyhulls. Results of studies by Batajoo and Shaver (1994) are generally supportive of these recommendations. They concluded that, for cows producing over 88 lb/day of milk, the diet should contain more than 30% NFC, but they found little benefit of a 42% over 36% NFC diet. Nocek and Russell (1988)

suggested that 40% dietary NFC was optimal in diets for lactating cows from an evaluation of diets based on alfalfa silage, corn silage, and 50:50 alfalfa:corn silage; dietary NFC ranged from 30 to 46%. Hoover and Miller (1991) regressed data from Nocek and Russell (1988) and demonstrated that when dietary NFC was greater than 45 to 50% or less than 25 to 30%, milk production was decreased. Alteration of dietary NFC has been shown to influence ruminal fermentation patterns, total tract digestion of fiber, and milk fat percentage (Sutton et al., 1987; Sievert and Shaver, 1993).

Hoover (West Virginia University, unpublished data), measured slow and fast sugar and starch (NSC) digestion of various nonforage fiber sources in situ and found quite a range in the rate of digestion (Kd) of these fractions. For example, the fast pool for beet pulp, representing 72% of the 15.9% total NSC as a percentage of DM, had a Kd of 429%/h, while brewer's grains (18.4% NSC as a percentage of DM) had a Kd for the fast pool (65% of NSC) of only 66%/h. The variation in composition and rate of the NSC fraction of various nonforage fiber sources can greatly affect overall fiber digestion through changes in rate of passage and amounts of indigestible residue remaining in the rumen.

In addition to total starch level, the rate and extent of ruminal starch digestion also may affect the amount of a particular starch source that can safely be added to a diet. The rate at which NFC is fermented and the retention time of the NFC in the rumen determine ruminal fermentation of NFC. Rate of fermentation of starch varies extensively by type of grain and processing. Herrera-Saldana et al. (1990) ranked the degradability of starch from various sources as follows: oats > wheat > barley > corn > milo. Processing methods, such as fine grinding and steam flaking, also may alter ruminal availability of starch. Lykos and Varga (1995) demonstrated that effective degradability of starch in situ for cracked corn, fine ground corn, and steam flaked corn was 44.4, 64.5, and 75.4%, respectively. In

addition, the effective degradability of starch was increased for ground versus cracked soybeans whether raw or roasted. Most grain processing methods increase both rate of starch fermentation and ruminal starch digestibility. It is important to note that grinding increases both rate of digestion and rate of passage, which have counteractive effects on ruminal digestibility (Gaylean et al., 1981). Animal characteristics and level of intake affect rate of passage. Therefore, fine grinding may have less effect on ruminal starch digestibility at higher levels of intake, such as dairy cattle in early lactation, than for growing or fattening animals, dry cows, or dairy cattle in late lactation.

Results of lactation studies comparing starch sources with differing digestibilities are somewhat inconsistent. Herrera-Saldana and Huber (1989) reported higher milk production with a barley-cottonseed meal diet than with a milo-cottonseed meal diet, while McCarthy et al. (1989) and Casper et al. (1990) found milk production to be higher in diets containing corn than those containing barley. Wilkerson and Glenn (1997) demonstrated increased yield of milk for cows fed high moisture corn versus dry corn (91.7 vs. 87.3 lb/d) and ground corn versus rolled corn (92.0 versus 87.1 lb/d). Lykos et al. (1997) demonstrated that increasing rate of total NSC digestion from 6 to 7.9 %/h significantly increased milk yield 5.5 lb/day and protein yield 130 g/day. Aldrich et al. (1993) observed lower 4% FCM when diets high in rapidly fermentable NSC were fed to lactating cows in early lactation. Seymour et al. (1993) demonstrated that cows fed with partially pelleted steam flaked grain produced less milk compared those fed pelleted grain. Diets with increased ruminally degraded starch did not affect milk yield or FCM in other studies (Oliveira et al., 1995). Some of the variation in results of type and amount of NFC in the diets for high producing lactating cows may be related to: 1) effects of rapidly degradable starch on ruminal digestion of fiber, which can decrease the differences between diets relative to total carbohydrate digestion; 2) level to which

NFC replaces fiber in the diet, as this can effect rumination and saliva production; 3) site of starch digestion; 4) level of DM intake and physiological state of the animal; and 5) conservation and processing methods used to alter rate and extent of NFC digestion.

Nocek and Russell (1988) conducted starch analysis on a variety of grain and forage sources and the relationship between starch and NDF content was  $r = -0.68$  ( $P > 0.10$ ). In order to identify relationships between chemical carbohydrate measures and production performance in lactating dairy cows, Nocek (1988) assembled a database representing 14 studies consisting of 62 diets. Milk yield averaged 70 lb/day and DM intake averaged 46 lb/d. Neutral detergent fiber and starch intakes were poorly correlated ( $r = 0.36$ ,  $P > 0.10$ ), while starch was highly correlated to  $NE_l$  concentration of the diet ( $r = 0.85$ ,  $P < 0.01$ ). Starch intake was highly correlated to milk yield ( $r = 0.60$ ,  $P < 0.01$ ), DM intake, ( $r = 0.85$ ,  $P < 0.01$ ), and protein yield ( $r = 0.71$ ,  $P < 0.01$ ). In these studies, NDF was not correlated to milk or protein yield; however, a significant relationship between NDF intake and DM intake was observed ( $r = 0.69$ ,  $P < 0.01$ ). The studies evaluated by Nocek et al. (1988) were mainly diets where the starch sources were primarily barley or corn and with or without processing imposed on these two starch sources. Varga and Kononoff (1999) evaluated 16 studies from 1992 through 1998 where byproduct feeds were also included as well as processed feed sources, such as corn and barley. The relationship between NFC and milk yield was poor and tended to be curvilinear. More information is needed to understand the composition of the NFC that contributes to the dietary DM as more than likely rate of degradation has a major influence on its utilization by the animal and ultimately milk yield (Beauchemin et al., 1997; Lykos et al., 1997). More direct measures, such as starch, sugar, pectin, glucans, and volatile fatty acids, are needed to better formulate rations for dairy cows. Table 7 demonstrates how much feeds can

vary based on not only their concentration of NDF, starch, and sugars, but in particular, the amount digested. Data of this nature, along with rate of degradation, are needed to better formulate rations for lactating cows.

To emphasize the need for more information of feeds for soluble carbohydrates, as well as digestibility, 35 corn silage samples in the Capital region of PA were evaluated for various nutrient contents and digestibility estimates (Table 8). The starch content of these corn silage samples was not that different; however, starch digestibility varied by almost 20% units. This demonstrates that without knowing the extent of starch digestion, a starch value on its own may not be that meaningful.

### *Fructans*

Fructans (fructosans) are water-soluble chains of fructose found in the cell contents of plants. They are the principal storage carbohydrates of temperate cool season grasses. Depending on the species and environmental conditions, temperate grass forage has been reported to contain less than 1% and up to 30% fructan (Smith, 1973). Although mammals can utilize fructose, they do not have the enzymes to digest fructans (Nilsson et al., 1988). In the rumen, both bacteria and protozoa ferment fructan (Ziolecki et al., 1992). Fructans can be fermented to lactic acid during ensiling (Muller and Lier, 1994) and in the rumen (Ziolecki et al., 1992). Additionally, rumen microbes can degrade fructan and store it as starch and utilize it at a later time when other nutrients are no longer available.

### *$\beta$ -Glucans*

The  $\beta$ -glucans are found in the endosperm and cell walls of grasses (Van Soest, 1994). Barley and oats are major sources of  $\beta$ -glucans, containing from 4 to 12% by weight. Just as mammals cannot digest cellulose, they also cannot digest  $\beta$ -glucans. However, this

carbohydrate appears to be very rapidly fermented in the rumen. In steers fed barley, ruminal in sacco disappearance of  $\beta$ -glucans varied by lot of barley but was between 61.4 and 70.4% of DM at time 0 and 93.8 to 96.2% of DM by 8 hours. Disappearance of  $\beta$ -glucan was greater from dry rolled than from steam rolled barley (Engstrom et al., 1992).

### *Pectic Substances*

Pectic substances are found chiefly in the middle lamella of the plant cell wall and have been defined as non-starch polysaccharides soluble in water, in chemicals which remove divalent cations (eg.,  $\text{Ca}^{++}$ ,  $\text{Mg}^{++}$ ), and in dilute acids or bases that break covalent bonds (Doner, 1986). In terms of sugar composition, they contain a galacturonic acid backbone with rhamnose inserts, plus neutral sugar side chains made up largely of arabinose and galactose (Jarvis, 1984). There are differences among plants and plant parts in the content and composition of pectic substances (Hall, 2000). Because they are very complex carbohydrates, analysis for pectic substances has been difficult, and there are few values available (Hall, 2000). By-products, such as citrus pulp, sugar beet pulp, and soybean hulls, are reported to contain 29, 33.7, and 20% pectin, respectively. Among forages, grasses are low in pectic substances (2 to 5%), while legumes contain higher quantities (7 to 14%) (Chesson and Monro, 1982).

Pectins are rapidly and extensively degraded by rumen microbes. In vitro fermentation rates of 30 to 40% per hour for pectin have been reported (Hatfield and Weimer, 1995). Pectin fermentation tends to produce high acetate to propionate ratios and relatively little or no lactate. However, the organic acid contribution from the fermentation of pectic substances depends on its sugar composition (Titgemeyer et al., 1992). The yield of microbes from pectin or pectic substances is not different from starch (Strobel and Russell, 1986). However, the fermentation of pectin at low pH

(5.8) is reduced, resulting in a lower extent of degradation and up to 70% less microbial protein produced. A more acidic ruminal pH translates into decreased amounts of pectin fermented in the rumen (Ben-Ghedalia et al., 1989) and a decrease in the amounts of microbial protein and VFA available to the animal. Unlike starch, the cow cannot digest pectic substances that escape the rumen.

Lactating cows fed diets formulated to contain a greater proportion of by-product feeds (citrus pulp and beet pulp), as compared to those containing more starch (from corn products), had lower intakes (Solomon et al., 2000), decreased milk protein percentage and yield (Mansfield et al., 1994; Solomon et al., 2000; Leiva et al., 2001), and increased milkfat percentage (Mansfield et al., 1994; Leiva et al., 2001). Mertens et al. (1994) compared the efficacy of carbohydrate sources in utilizing alfalfa silage non-protein nitrogen (NPN) with cows fed diets containing 19% citrus pulp plus 19% high moisture shell corn, or 39% high moisture shell corn (% of dietary DM), with or without supplementation with expeller soybean meal. The cows on the citrus diets showed greater milk and protein yield responses to supplemental rumen escape protein than did cows on the high moisture corn diets, suggesting a poorer efficacy of NPN utilization with citrus.

It appears that altering the proportions of sugars, starch, and soluble fiber in rations can alter intake, milk yield and composition, and feed efficiency. In a study designed to examine the microbial yield from the different NFC, isolated bermudagrass NDF (iNDF), and blends of sucrose, citrus pectin, or corn starch and iNDF were fermented in vitro (Hall and Herejk, 2001). The results show that the maximal yield of CP was greatest from the starch fermentation (32.5 mg), and lower, but similar, between pectin (28.1 mg) and sucrose (27.6 mg). If the lower yields of CP from the fermentations of sucrose and pectin translate to reduced amino acid amounts available to the cow, it could explain the



reductions in milk protein in the animal feeding trials. For pectin, it could explain the lower efficacy of NPN utilization and the response to feeding rumen escape protein.

### Summary

To minimize the risk of ruminal acidosis and optimize the provision of rapidly fermented carbohydrate in the ration of dairy cows, a balance of NFC types is recommended. The 2001 NRC recommended making adjustments for NFC in the ration along with NDF and forage NDF as a percentage of DM (Table 9). Suggestions for target levels of NFC to be included in rations (% of DM) are: 5% sugars, 10% soluble fiber, and 20% starch (Hall, 2000). If pectin/soluble fiber sources and sugars are included in rations as the predominant soluble carbohydrate sources, then additional rumen undegradable protein may be recommended. Starch appears to offer the greatest potential to optimize microbial protein synthesis; however, feeding high levels of starch can lead to ruminal acidosis and digestive upset. Information is needed to determine what extent sugars and starches are interchangeable to deliver glucose to the small intestine and what proportions of soluble fiber, sugars, and total effective NDF to include in rations to prevent digestive upsets.

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**Table 1.** Neutral detergent soluble carbohydrate of selected feedstuffs and their fermentation end products.

Feed	Major NFC Fractions <sup>1</sup>	End-products
Alfalfa hay	Pectin and sugar	Acetate and Lactate
Alfalfa silage	Pectin, sugar, and organic acids	Acetate, Lactate, Butyrate
Corn silage	Starch and organic acids	Lactate
Grass hay	Fructan and sugar	Lactate
Grass silage	Organic acids and sugar	Lactate
Corn grain	Starch	Lactate
Barley grain	Starch and b-glucans	Lactate and Acetate
Wheat grain	Starch and b-glucans	Lactate and Acetate
High moisture corn	Starch and organic acids	Lactate
Hominy	Starch	Lactate
Molasses	Sugar	Lactate
Whey	Sugar	Lactate
Beet pulp	Pectin	Acetate
Citrus pulp	Pectin and sugar	Acetate and Lactate
Almond hulls	Pectin and sugar	Acetate and Lactate
Soy hulls	Pectin	Acetate
Wheat midds	Starch	Lactate

<sup>1</sup>NFC = nonfiber carbohydrates.

**Table 2.** Composition of the neutral detergent soluble carbohydrates (NDSC) of selected feedstuffs.<sup>1</sup>

Feedstuff	sugar	starch	pectin	VFA <sup>2</sup>
	-----% of NDSC-----			
Alfalfa silage	0	24.5	33.0	42.5
Grass hay	35.4	15.2	49.4	0
Corn silage	0	71.3	0	28.7
Barley	9.1	81.7	9.2	0
Corn grain	20.9	80.0	0	0
Beet pulp	33.7	1.8	64.5	0
Soyhulls	18.8	18.8	62.4	0
Soybean meal, 48% CP	28.2	28.2	43.6	0

<sup>1</sup>Adapted from Hoover and Miller (1990).

<sup>2</sup>VFA = volatile fatty acids.

**Table 3.** Estimated rumen fermentation characteristics.<sup>1</sup>

	Proportion of carbohydrates converted to substrate				
	Diet <sup>2</sup>	Acetate	Propionate	Butyrate	Valerate
Soluble CHO <sup>3</sup>	R	0.69	0.20	0.10	0
	C	0.45	0.21	0.30	0.04
Starch	R	0.59	0.14	0.20	0.06
	C	0.40	0.30	0.20	0.10

<sup>1</sup>From Van Soest, 1994.

<sup>2</sup>R codes roughage diets: C codes diets containing more than 50% of a cereal-based concentrate diet.

<sup>3</sup>Soluble carbohydrate (**CHO**) fraction includes organic acids and pectin.

**Table 4.** Least squares means of dextran yield at fermentation hour 4 and its proportion of sucrose initially added to the vial (Hall, 2002).

Sucrose, mg <sup>1</sup>	Dextran, mg	SE <sup>2</sup>	For Hour 4, Dextran/ Initial Sucrose In Vial
65	9.3	1.59	0.14
130	11.8	1.59	0.09
195	15.0	1.59	0.08

<sup>1</sup>Milligrams of sucrose added per fermentation vial.

<sup>2</sup>Standard error.

**Table 5.** Least squares means  $\pm$  standard error of organic acid production at the hour of their peak yields (millimolar in 32 milliliters) (Hall, 2002).

Sucrose, mg <sup>1</sup>	Total	Acetate 24 hour	Propionate 24 hour	Butyrate 24 hour	Lactate 4 hour
	Organic Acids 24 hour				
65	25.8 $\pm$ 1.9	15.9 $\pm$ 1.2	7.4 $\pm$ 0.6	2.4 $\pm$ 0.2	0.12 $\pm$ 0.83
130	39.6 $\pm$ 1.9	22.7 $\pm$ 1.2	12.8 $\pm$ 0.6	4.0 $\pm$ 0.2	1.75 $\pm$ 0.83
195	53.8 $\pm$ 1.9	29.9 $\pm$ 1.2	17.6 $\pm$ 0.6	6.0 $\pm$ 0.2	4.82 $\pm$ 0.83

<sup>1</sup>Milligrams of sucrose added per fermentation vial.

**Table 6.** Changes in milk yield and composition with changes in sucrose and starch supplementation (Broderick et al., 2000).

Sucrose, %	Starch, %	DM intake, lb	Milk, lb/day	Milk Fat, lb/day	Milk Protein, lb/day	FCM, lb/day
0	7.5	54.0	85.8	3.24	2.73	89.3
2.5	5.0	56.4	89.1	3.37	2.82	93.0
5.0	2.5	57.3	88.2	3.64	2.84	96.8
7.5	0	57.3	86.9	3.57	2.82	95.2

**Table 7.** Fermentability of NDF, starch, and sugar for various feedstuffs (Hoover and Miller, 1990).

Ingredient	NDF, %	NDF dig., %	Sugar + starch, %	Sugar + starch dig., %
Beet pulp	48	60	12.8	70
Hominy	23	45	54	65
Soyhulls	66	56	5.3	70
Wheat bran	33	58	47	80
Wheat midds	42	60	32	80

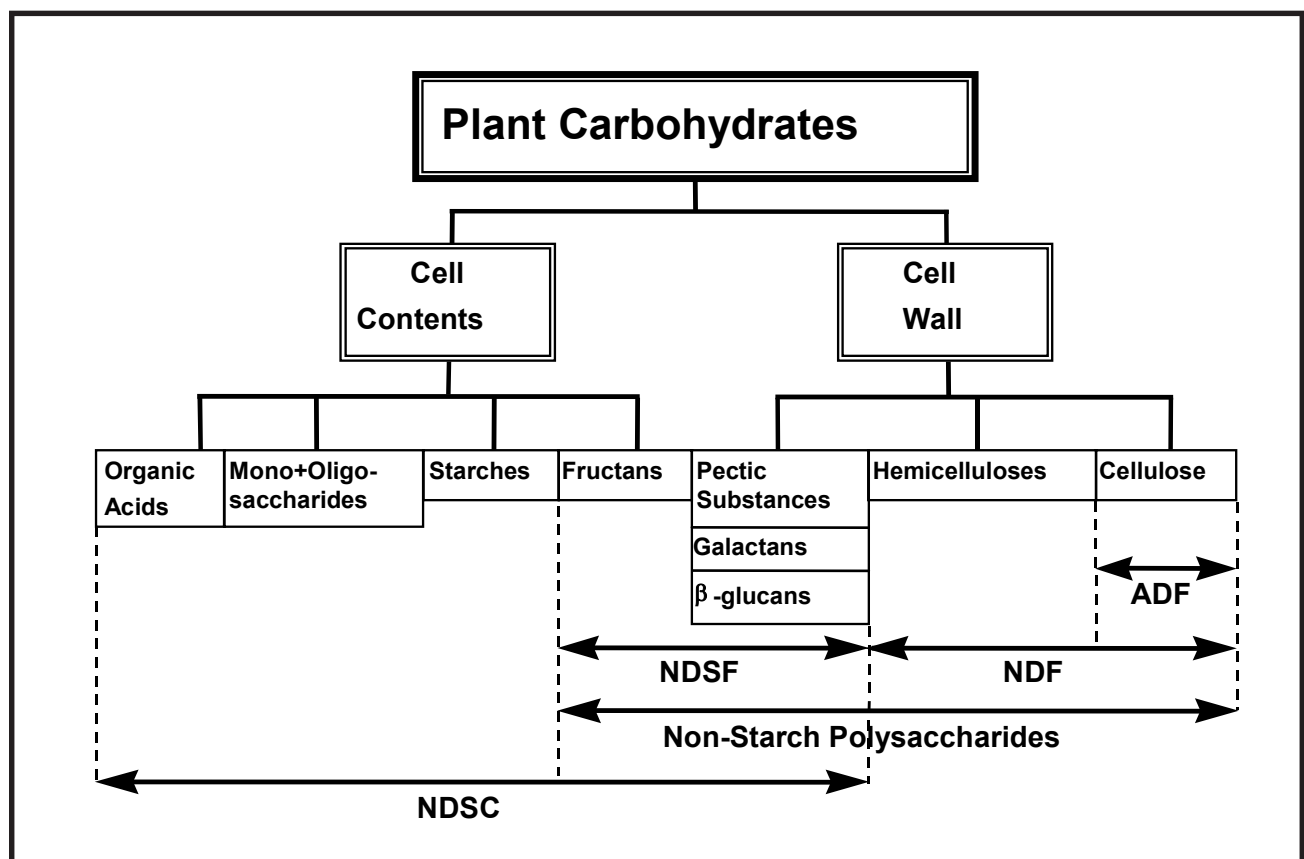
**Table 8.** Starch, starch digestibility, and DM comparison of corn silage samples (n = 35).

Starch, %	Starch digestibility, %	Dry matter, %
34.4	75.0	41.8
33.5	74.6	42.0
27.2	78.3	39.8
31.2	91.2	34.7
31.2	93.5	30.7
30.4	88.3	33.8

**Table 9.** Minimum concentrations (% of DM) of total and forage NDF and maximum concentrations (% of DM) of NFC (NRC, 2001).<sup>1</sup>

Min forage NDF	Min NDF	Max NFC	Min ADF
19	25	44	17
18	27	42	18
17	29	40	19
16	31	38	20
15	33	36	21

<sup>1</sup>Values in this table are based on the assumption that actual feed composition has been measured. NFC = non fiber carbohydrates.



**Figure 1.** Plant carbohydrate fractions. The NDSC contain the carbohydrates listed and others of the appropriate solubility. ADF = acid detergent fiber, glucans = glucans, mono- + oligosaccharides = “sugars”, NDF = neutral detergent fiber, NDSC = neutral detergent-soluble carbohydrates, and NDSF = neutral detergent-soluble fiber. From Hall, 2000.





## New Developments in TMR Particle Size Measurement

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### Abstract

The Penn State TMR and Forage Particle Separator (**PSPS**), has become a routine technique used in forage and TMR particle size evaluation. The objective of this paper is to outline the procedure to estimate particle size using the PSPS and to briefly describe how it may be used to further understand rumen fermentation and feeding behavior. Although proven useful, a large proportion of smaller TMR particles may pass through both sieves of the original device. Recently, a smaller sieve measuring 1.18-mm has been added to improve accuracy of estimating the smaller particle fraction. The PSPS is also a useful tool to monitor sorting behavior of dairy cattle and can be used to troubleshoot a variety of feeding, digestion, metabolic, and production-related problems. Plotting the particle size distribution of both TMR and refusals using a newly developed spreadsheet can also be useful in evaluating the degree of sorting behavior exhibited by dairy cows.

### Introduction

Since its introduction in 1996, The PSPS, has become a routine technique used in forage and TMR particle size evaluation. The rapid acceptance of this method is due to the simplicity of the procedure, low cost of analysis, and rapid determination of results. The PSPS was originally constructed out of two sieves and

a bottom pan. Apertures of the two sieves measured 19.0 and 8.0-mm and had thickness of 12.2 and 6.4-mm. With its simple construction and size, the PSPS sieving method can be frequently implemented on the farm and used at the time of harvest or feeding to determine particle size of forages or TMR (Lammers et al., 1996). Even though the apparatus has been widely accepted and particle size measurements using the PSPS are now commonly reported in the scientific literature, the TMR typically contain 40 to 60 % concentrate, most of which passes through the smallest sieve measuring 8.0-mm sieve. Recently, a smaller sieve measuring 1.18-mm has been added to improve accuracy of estimating the smaller particle fraction. The purpose of this paper is to outline some of the recent developments and understandings in the TMR particle size measurement as estimated by the PSPS.

### Using The Penn State TMR and Forage Particle Separator

#### *Sieving Frequency*

The PSPS must be operated correctly in order to achieve accurate results. During our field experience, we have observed that the rate of shaking, or frequency of moving the device, sometimes differs between users. Because the frequency of shaking will influence particle movement over enclosed sieves and will ultimately affect particle size estimates, we have

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recently tested the effect of shaking frequency on TMR particle size measurement (Kononoff et al., 2003). The unit used to express frequency of movement is a Hertz (**Hz**), and one Hertz is equal to the number of full forwards and backward movements within one second. For example, if the PSPS is shaken at a frequency so that a total of 66 full movements (forward and back) would occur within 60 seconds, the measured frequency would be 1.1 Hz. In the experiment, three frequencies, 0.9, 1.1 and 1.6 Hz (slow, medium, and fast), were used to determine the effect of sieving frequency on TMR particle size estimation.

Results of the experiment are listed in Table 1 and indicate that reducing sieving frequency below 1.1 to 0.9 Hz resulted in significantly more material being retained on the 19.0-mm sieve and less on the 8.0 and 1.18-mm sieves. Consequently, these results yield a mean particle length (**MPL**) that was significantly greater when estimated at 0.9 Hz compared to 1.1 Hz. In contrast, increasing sieving frequency from 1.1 to 1.6 Hz did not result in significant differences in particle size measurements. We recommend the PSPS to be shaken at 1.1 Hz (66 cycles/min) or greater. Practically, it is important to understand that one cannot shake the PSPS too fast; however, movements which are too slow may result in very different estimates of particle size. It is recommended that operators of the device calibrate the frequency of movement over a distance of 17 cm for a specified number of times. The number of full movements divided by time in seconds results in a frequency value that can be compared to this recommendation.

#### *Effect of Moisture on Particle Size Measurement*

We know that sample moisture content may affect sieving properties, but it is not practical to recommend that particle size estimation be conducted at a standardized moisture content (Finner et al., 1978). The PSPS

is designed to describe particle size of the feed offered to the animal, thus samples should not be chemically or physically altered before sieving. Because sample moisture loss may occur during storage or transport, a study was carried out to determine the effects of forage moisture on particle size measurement, as estimated by the PSPS. In the experiment, samples of both alfalfa haylage and corn silage were placed in a large forced air oven set at 55 °C. Five times over a 48 hour period, samples of different moisture content were removed from the oven and analyzed for particle size using the PSPS.

Tables 2 and 3 outline the effects of forage moisture content on particle size estimated for both alfalfa haylage and corn silage. For alfalfa haylage, oven drying times of 0, 2, 6, 12, and 48 hours resulted in moisture concentrations of 57.4, 35.6, 10.4, 2.5, and 0.0 %, respectively. Similarly, for corn silage, oven drying times of 0, 3, 6, 18, and 48 hours resulted in moisture concentrations of 58.0, 34.4, 14.6, 3.5, and 0.0 %, respectively. For alfalfa haylage samples, particle size measurements were not significantly different between 57.4 and 35.6 % moisture, indicating that moisture loss in samples within this range will not affect particle size measurements. Conversely, for corn silage, the amount of particle mass < 1.18-mm was significantly different between 58.0 and 34.4 % moisture and resulted in a small but significant difference in MPL. These results suggest that moisture loss from corn silage may affect particle size results; but these differences, when observed, are small. For alfalfa haylage, compared to 57.4 and 35.6% moisture, the amount of material > 19.0-mm was significantly lower in samples containing 10.4, 2.5, and 0.0% moisture, but this difference was not observed for corn silage as most material > 19.0-mm contained cob particles for which size measurement appeared to be unaffected by moisture content. For both forages, amount of material < 1.18-mm was greatest at 0.0 % moisture content, while MPL decreased with

decreasing moisture content. These results are similar to Finner et al. (1978) who suggested that completely drying a sample results in shattering of particles and further size reduction during the sieving process. Differences in sieving results associated with sample drying may have been due to brittle particles that shatter during shaking or to decreased adhesion of small particles to larger ones when materials are dry.

Because it would be impractical to recommend a constant sample moisture for measuring forage or TMR particle size during field measurement, it is advantageous to know that slight losses of moisture have only limited effects on measurements according to the moisture range of this study. It is recommended that samples be analyzed in the same physical form as that fed to the animal and moisture loss in samples should be minimized. Based on our results, only small differences result when sample moisture loss is as much as 40 % of the original sample.

### **Particle Size Recommendations**

Table 4 contains the original particle size recommendations of corn silage, haylage, and TMR according to the Penn State Technical Bulletin, DAS 96-20 (Heinrichs, 1996). Although proven useful, the basis for the recommendations was simple surveys and field observations. More recently, a number of experiments have been conducted that have attempted to further understand the effects of forage and TMR particle sizes, thus these recommendations have been revised and are listed in Table 5 and published in the newly revised Penn State Technical Bulletin, DAS 02-42 (Heinrichs and Kononoff, 2002).

#### *TMR Particle Size and Chewing Activity*

It is widely understood that dairy cattle require fiber in a coarse physical form because it is effective in stimulating chewing activity, saliva secretion, and maintaining normal rumen

health and function. As a result of this understanding, many studies have attempted to manipulate forage particle size to increase chewing activity and thereby increase the buffering capability in the rumen.

Recently, Krause et al. (2002a,b) demonstrated that feeding an alfalfa silage based TMR with approximately 23% greater than 19.0-mm, increased total chewing time 7.5 min/kg of DM consumed compared to a TMR which contained less than 1% greater than 19.0-mm. Similarly, Kononoff and Heinrichs (2003a) noted that increasing particle size of an alfalfa haylage based TMR from 3 to 31% of the TMR greater than 19.0-mm increased chewing activity 6.7 min/kg of DM consumed. Taken together, these studies support the suggestion that the proportion of material retained on the top sieve of the PSPS is positively correlated to chewing activity (Krause et al., 2002b).

Differences in total time spent eating and ruminating are not always observed when feeding corn silage of different particle size. Bal et al. (2000) observed that fine cut length reduced eating activity but did not affect time spent ruminating; however, this has been reported by others (Schwab et al., 2002). In studies in which feed refusals were chemically analyzed (Bal et al., 2000; Kononoff and Heinrichs, 2003b), diets of longer particle size resulted in a higher concentration of NDF, indicating greater tendency for sorting. Based on these and other studies, it is apparent that increasing particle size of a TMR can increase chewing activity; however, if the TMR is too long, this may promote sorting behavior.

#### *TMR Particle Size and Rumens pH*

Nutritionists are often concerned about rumen pH because when pH levels fall below 6.0, fiber digestion may be impeded (Russell and Wilson, 1996). Generally speaking, rumen pH is a function of lactic acid and VFA production and is buffered by saliva (Maekawa

et al., 2002). Because of this idea, it is common practice to feed diets of longer particle size and greater amounts of effective fiber so that saliva production is stimulated. In support of this understanding, Krause et al. (2002b) noted that intake of particles > 19.0-mm was negatively correlated to the amount of time rumen pH spent below 5.8. However, we also know that forage should not be harvested at excessively long lengths. Kononoff and Heinrichs (2003a) demonstrated that if diets are too long, eating patterns may be affected and may result in lower mean rumen pH even though chewing activity may be increased. In this study, increasing the proportion of particles > 19.0-mm from 3 to 12% did result in increased chewing activity and rumen pH. However, increasing the proportion of particles to 31% resulted in depressions in mean rumen pH, which was thought to be a result of changes in eating behavior and increased sorting.

In light of these studies, it is apparent that particle size can affect rumen pH; however, differences in particle size do not always result in differences in mean rumen pH. When evaluating a diet to determine a possible risk of subclinical acidosis, we should review levels of fiber and non-structural carbohydrates, along with their associated fermentability (Yang et al., 2001a).

#### *New Particle Size Recommendations*

Our original particle size recommendations have been revised, and the amount of TMR > 19.0 mm has been reduced from the original recommendation. This is because literature demonstrates that when rations are correctly balanced for both fiber and nonstructural carbohydrates, no severe deleterious effects on rumen pH from reduced chewing activity are observed when rations contained less than 6% of the particles > 19.0 mm. Although many rations are commonly fed with longer particles, there is increasing evidence that these rations may result in increased sorting.

The recommended amount of material between 8.0 and 19.0 mm was maintained and reflected the variation commonly observed on commercial dairy farms (Heinrichs et al., 1999). As a result of the modification of the PSPS, particle size recommendations of material < 8.0 mm is now further partitioned into the proportion between 1.18 and 8.0 mm and the proportion < 1.18 mm. Given that no severe negative effects have been observed in animals consuming rations which contained 30 to 50 % of the ration 1.18 to 8.0 mm, our recommendations are limited to that range (Kononoff and Heinrichs, 2003 a,b).

Few published studies have included the new sieve in particle size measurements; however, it is generally understood that particles retained on a 1.18-mm sieve pass out of the rumen slower than those not retained (Poppi et al., 1985). Recently, using a different method of dry sieving, Yang et al. (2001b) demonstrated that rumen outflow rate of particles less than 1.18-mm averaged 5.57%/h compared to those retained on a 3.35-mm sieve, which had an average outflow rate of 1.75%/h. Thus, the additional sieve should prove useful in further understanding of factors affecting rate of passage. Although the proportion of material < 1.18 mm was not different within two published experiments, no negative effects were observed when as much as 20% of the material was less than 1.18 mm (Kononoff and Heinrichs, 2003 a,b). We are thus currently limited to recommend that TMR contain no more than 20% of the material < 1.18 mm. This recommendation may require further refinement based on the relative amounts of soluble and degradable carbohydrate components in the TMR.

#### **Understanding Eating Behavior by Using the PSPS**

##### *Rations That Promote Sorting Behavior*

Particle size analysis is only part of the story. Dairy rations must be balanced for proper

nutrient intake, and as we all know, there is no standard ration. Given a ration, which appears to be adequate in terms of its chemical composition, particle size results can aid in our understanding of how nutrients contained in the ration will be consumed by the animal. Further understanding of how particle size affects feeding behavior is warranted because if the ingestion of feed is more or less uniform, rumen fermentation will proceed with limited fluctuation in acid production. Alternatively, if ingestion is selective, fluctuations in ruminal acid production may result in imbalance, leading to abnormal or pathological conditions in the rumen (Van Soest, 1994). As a result of this, when attempting to identify limits to production, particle size evaluation should be included in our trouble shooting procedures.

Lets look at an experimental situation in which lactating Holstein cows were fed one of two different rations that were chemically identical but one considered “long” and the other shorter but adequate according to our current guidelines. As presented in Table 6, the long ration contained 16 % of the particles greater than 19.0-mm while the short ration contained 7% of the particles > 19.0-mm. In both rations, the proportions of particles less than 1.18-mm were similar, approximately 4%. The cumulative percentage of material that falls through each sieve (cumulative percent undersize) is also presented in Table 6 and will be used for graphical purposes to be illustrated later. What can these measurements tell us about possible animal feeding behavior? In order for a ration, which looks good on paper, to work, it must be consumed. There is increasing understanding that the amount of particles >19.0 mm is correlated with eating time and chewing behavior (Johnson et al., 2003). It is likely that this relationship is not perfectly linear, but as ration particle size increases, total chewing time should also increase, however, only up to a point. Past this point, animals may then exhibit selective tendencies against longer, high fiber containing particles. This is cause for concern

because the ration was balanced with understanding that the animals would consume these longer particles.

### *Refusal Particle Size and Fiber Content*

Now lets evaluate the particle size of the remaining refusal after the animals have had a 24-hour access to the feed. Remember, we want the refusals to be similar to the feed originally fed, if its drastically different (> 5% variation), animals may have sorted out the ration and consumed something different that what we originally assumed when we balanced the ration. As seen in Table 7, the particle size of the long TMR is much longer than originally offered. From these observations, we can see that animals consuming the long ration consumed more grain as 1% of the DM was less than 1.18-mm, compared to 5% when animals consumed the short ration.

To support our observation that animals consuming the long ration exhibited an increased sorting tendency, we can evaluate the NDF content of the feed remaining in the bunk at 8, 16, and 24 hours after feeding. In our current example, the concentration of the original TMR for both long and short TMR was identical, 33% of DM. Data presented in Table 8 demonstrate that the NDF contents of the refusals at 24 hours after feeding for animals consuming the long TMR were 36 and 44% for the short and long TMR, respectively. After 24 hours for the long TMR remaining in the bunk, it was 11 percentage units greater in NDF than that originally fed, indicating considerable sorting activity. In comparison, the NDF content of the short feed remaining in the bunk was measured at 34, 36, and 36% at 8, 16, and 24 hours after feeding. This was very similar to the ration containing 33% NDF, indicating that very little sorting activity occurred. These data support our idea that particle size may affect how cows consume the ration and that feeding a ration that is too long or coarse may result in large amounts of sorting activity.

### *Using a Spread Sheet to Compare the TMR and Refusals*

Additional tools and guidelines to be used in the evaluation of particle size are becoming available. A simple spreadsheet that performs all of the calculations, while also graphing the results, can be downloaded from the Penn State Dairy Nutrition website <http://www.das.psu.edu/dcn/catforg/particle>. This spreadsheet can be used to gather information, while at the same time aid in further understanding feeding behavior. The size distribution of forage and TMR particles does not follow a normal distribution pattern; however, it can be plotted as a straight-line distribution using lognormal graphing paper. When plotting the data, sieve size is plotted on the horizontal or X-axis, while the cumulative percentage of material that falls below each sieve is entered on the vertical, or Y-axis. Once entered into the PSPS spreadsheet, these values are then plotted, and a best-fit line is drawn between each of the three points. For example in Figure 1, approximately 16% of the feed is more than 19.0-mm (0.75 inches), and as a result, 84% of the material is undersized. With 50% being retained on the 8.0-mm (0.31 inches) sieve, a cumulative amount of 34% falls below 8.0-mm. Lastly, 23% is retained on the 1.18-mm sieve, and as a result, only 5% now falls less than 1.18-mm (0.05 inches).

Figures 1 and 2 illustrate the differences in particle size between the originally fed TMR and the refusals. In both figures, the original TMR particle size is plotted with dark points and a best-fit line is drawn in the same color (upper line). Refusal particle size is plotted with lighter points and a best-fit line is drawn in the same color (lower line). Generally, we would desire that each point remain in, or close, to the recommended rectangles and that the associated best-fit lines be very close together. When rations in which little sorting has occurred, such as that illustrated in Figure 1, points falling on the same X-axis will be close together. In rations that are

severely sorted, as is Figure 2, the lighter refusal line is much lower than that of the original TMR line. To determine if sorting has occurred, compare the distance between the two points at each particle size (i.e. 1.18, 8.0, and 19.0-mm). In rations which result in a high degree of sorting, a greater spread between points will be observed.

Rations typically contain 40 to 60% concentrate, most of which passes through the sieve measuring 8.0-mm. Thus, the additional sieve will allow us to more precisely describe particle size by partitioning this fraction with the smaller sieve. Secondly, in Figure 1, the distance between the original TMR and refusal at >19.0-mm illustrates that some sorting of the material > 19.0-mm, but the proportion of particles < 1.18-mm was almost identical. This information indicated that although some sorting activity occurred, especially with respect to those particles > 19.0-mm, the proportion of grain in the TMR and refusal was similar. In comparison, Figure 2 illustrates that the proportion of particles of all size ranges were different, including those less than < 1.18-mm. Thus by including the additional sieve into our evaluation, we can more clearly see that the longer ration resulted in even greater selective tendency for the smaller grain particles.

### **Summary**

The PSPS is a tool to quantitatively estimate forage and TMR particle size. As with any diagnostic tool, users of the PSPS must ensure correct operation. Because differences in the rate of shaking, or frequency of moving the device, may affect particle size estimations, we specify that the PSPS be shaken at a frequency of 1.1 Hz or greater (66 cycles/min) with a stroke length of 17 cm. Recently, we have also investigated the effects of sample moisture on measurements and determined that small amounts of moisture loss from collected samples may affect particle size results, but these differences, when observed, are small. Conversely, completely drying a sample resulted

in large differences in particle size results. As a result of these observations, we recommend that samples be analyzed in the same form as fed to the animal, but precautions should be made so that moisture loss is minimized. In addition to these analytical recommendations, we have discussed the importance of particle size evaluation and outlined how the additional sieve and available spreadsheet may lead to further understanding of rumen fermentation and feeding behavior.

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**Table 1.** Effects of sieving frequency on particle size measurements of TMR<sup>1</sup> samples as measured by the modified Penn State Particle Separator.

Particle size (mm)	Frequency (Hz) <sup>2</sup>			P - value
	0.9	1.1	1.6	
TMR <sup>1,3</sup>				
> 19.0	40.9 <sup>a</sup>	6.4 <sup>b</sup>	6.9 <sup>b</sup>	0.02
19.0 – 8.0	24.6 <sup>b</sup>	42.9 <sup>a</sup>	43.8 <sup>a</sup>	0.05
8.0 – 1.18	31.5	36.7	35.3	0.31
< 1.18	3.0 <sup>b</sup>	14.0 <sup>a</sup>	14.0 <sup>a</sup>	0.001
MPL (mm) <sup>4</sup>	11.2 <sup>a</sup>	5.8 <sup>b</sup>	5.7 <sup>b</sup>	0.01

<sup>1</sup>TMR containing 50:50 forage to concentrate ratio and 9.5% grass hay, 25.3% corn silage, and 14.6% alfalfa haylage (% of DM).

<sup>2</sup>Means in the same row with different superscripts differ ( $P < 0.05$ ).

<sup>3</sup>46.0 + 1.6 % moisture.

<sup>4</sup>MPL = geometric mean length as calculated by the ASAE (2001).

**Table 2.** Effects of alfalfa haylage moisture content on particle size measurements according to the Penn State Particle Separator shaken at 1.2 Hz with a stroke length of 17 cm.

Particle size (mm)	Percent Moisture <sup>1</sup>					SEM <sup>2</sup>	P - value
	57.4	35.6	10.4	2.5	0		
> 19.0	61.5 <sup>a</sup>	63.0 <sup>a</sup>	45.2 <sup>b</sup>	40.3 <sup>b</sup>	27.5 <sup>c</sup>	2.87	< 0.001
19.0 – 8.0	25.3 <sup>c</sup>	24.4 <sup>c</sup>	35.4 <sup>b</sup>	37.3 <sup>a,b</sup>	44.5 <sup>a</sup>	2.25	< 0.001
8.0 – 1.18	11.3 <sup>d</sup>	10.6 <sup>d</sup>	15.1 <sup>c</sup>	18.0 <sup>b</sup>	22.6 <sup>a</sup>	0.69	< 0.001
< 1.18	1.9 <sup>c</sup>	2.1 <sup>c</sup>	4.3 <sup>b</sup>	4.4 <sup>b</sup>	5.4 <sup>a</sup>	0.27	< 0.001
MPL (mm) <sup>3</sup>	17.7 <sup>a</sup>	17.9 <sup>a</sup>	13.7 <sup>b</sup>	12.6 <sup>b</sup>	10.3 <sup>c</sup>	0.54	< 0.001

<sup>1</sup>Means in the same row with different superscripts differ ( $P < 0.05$ ).

<sup>2</sup>SEM = standard of mean.

<sup>3</sup>MPL = geometric mean length as calculated by the ASAE (2001).

**Table 3.** Effects of corn silage moisture content on particle size measurements according to the Penn State Particle Separator shaken at 1.2 Hz with a stroke length of 17 cm.

Particle size (mm)	Percent Moisture <sup>1</sup>					SEM <sup>2</sup>	P - value
	58.0	34.4	14.6	3.47	0		
> 19.0	14.3	11.0	9.5	9.6	12.9	2.16	0.32
19.0 – 8.0	74.0 <sup>a</sup>	74.5 <sup>a</sup>	73.2 <sup>a</sup>	70.4 <sup>a</sup>	52.3 <sup>b</sup>	2.08	< 0.001
8.0 – 1.18	11.4 <sup>d</sup>	13.1 <sup>c,d</sup>	15.4 <sup>b,c</sup>	18.0 <sup>b</sup>	31.5 <sup>a</sup>	1.36	< 0.001
< 1.18	0.23 <sup>d</sup>	1.36 <sup>c</sup>	2.0 <sup>b</sup>	2.0 <sup>b</sup>	3.4 <sup>a</sup>	0.16	< 0.001
MPL (mm) <sup>3</sup>	12.1 <sup>a</sup>	11.2 <sup>b</sup>	10.6 <sup>b,c</sup>	10.2 <sup>c</sup>	8.62 <sup>d</sup>	0.33	< 0.001

<sup>1</sup>Means in the same row with different superscripts differ ( $P < 0.05$ ).

<sup>2</sup>SEM = standard error of mean.

<sup>3</sup>MPL = geometric mean length as calculated by the ASAE (2001).

**Table 4.** Original recommended forage and TMR particle size recommendations as measured by the Penn State Particle Separator (Heinrichs, 1996).

Percentage retained	Type		
	Corn Silage <sup>1</sup>	Haylage <sup>2</sup>	TMR
> 19.0 mm	2 - 4	15 - 25	6 - 10
19.0 – 8.0 mm	40 - 50	30 - 40	30 - 50
8.0 – 1.18 mm	40 - 50	40 - 50	40 - 60

<sup>1</sup>This recommendation applies if corn silage is not the sole forage used in the TMR; if cornsilage is chopped and rolled, 10 to 15% may be > 19.0-mm.

<sup>2</sup>This recommendation applies if haylage is contained in a bunker silo; if it is in a sealed silo, 10 to 15% may be > 19.0-mm.

**Table 5.** Forage and TMR particle size recommendations<sup>1</sup> based on two experiments that fed either alfalfa haylage or corn silage, with or without cottonseed hulls, to cows in early lactation.

Sieve Size	Type		
	Corn Silage	Haylage	TMR
	% of DM retained		
> 19.0 mm	5 ± 3	15 ± 5	5 ± 3
19.0 – 8.0 mm	55 ± 10	60 ± 15	40 ± 10
8.0 – 1.18 mm	40 ± 10	30 ± 10	40 ± 10
< 1.18 mm	< 5	< 5	< 20
MPL (mm) <sup>2</sup>	8 ± 2	10 ± 2	5 ± 2

<sup>1</sup>As measured by the Penn State Particle Separator (Heinrichs and Kononoff, 2002).

<sup>2</sup>MPL = geometric mean length as calculated by the ASAE (2001).

**Table 6.** Particle size measurements of one short and one long corn silage based TMR.

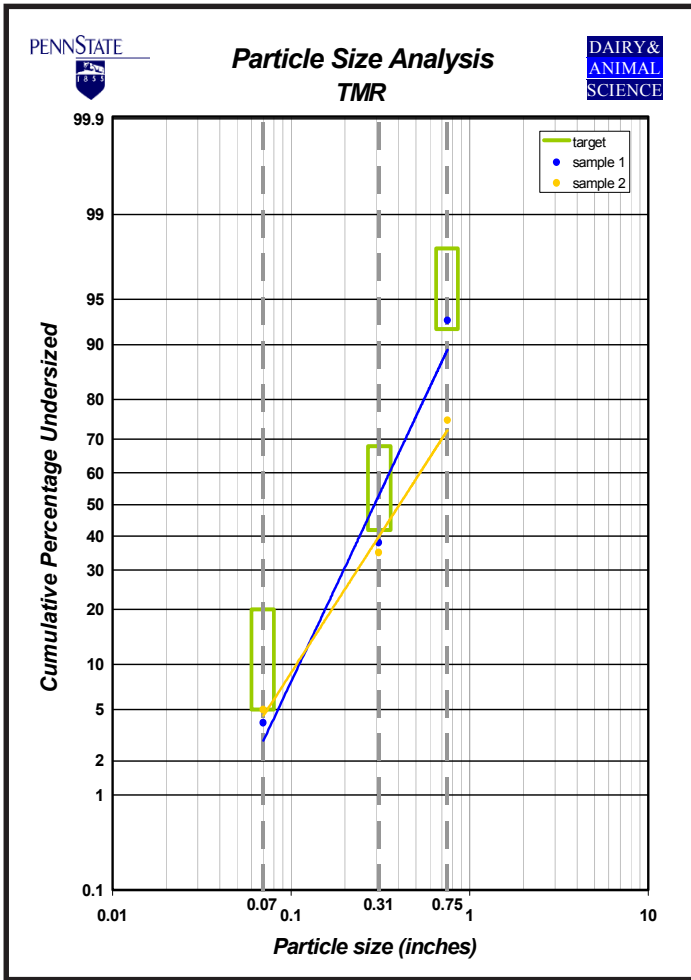
Sieve Number	Particle Size (mm)	Short TMR		Long TMR	
		Percent Retained	Cumulative Percent Undersized	Percent Retained	Cumulative Percent Undersized
1	> 19.0	7	93	16	84
2	19.0 – 8.0	56	37	50	34
3	8.0 – 1.18	34	4	30	4
4	< 1.18	4	-	4	-

**Table 7.** Particle size measurements of refusals for animals fed one short and one long corn silage based TMR.

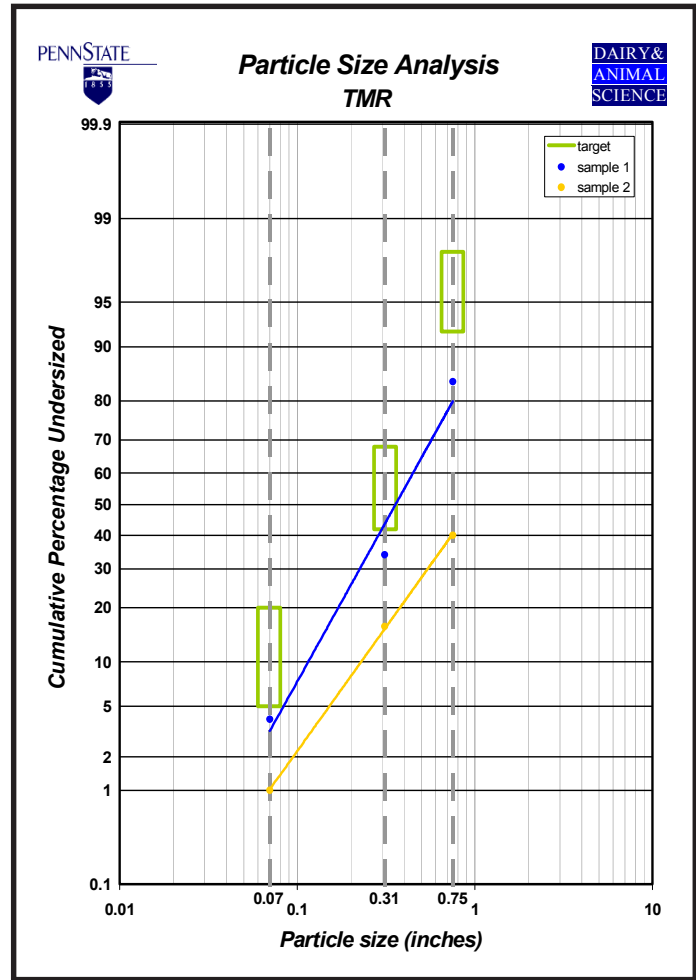
Sieve Number	Particle Size (mm)	Short TMR		Long TMR	
		Percent Retained	Cumulative Percent Undersized	Percent Retained	Cumulative Percent Undersized
1	> 19.0	25	75	60	40
2	19.0 – 8.0	40	35	24	16
3	8.0 – 1.18	31	4	15	1
4	< 1.18	5	-	1	-

**Table 8.** The effect of feeding two rations of different particle size on the NDF concentration of refusals.

Hours After Feeding	Short TMR	Long TMR
	% NDF (DM basis)	
0	33	33
8	34	35
16	36	41
24	36	44



**Figure 1.** Particle size distribution of short TMR (upper line) and refusals (lower line) as measured by the Penn State Particle Separator.



**Figure 2.** Particle size distribution of long TMR (upper line) and refusals (lower line) as measured by the Penn State Particle Separator.

## Problems with Sorting in Total Mixed Rations

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### Abstract

Cows need to consume physically effective fiber to maintain health. In order for this to happen, cows must be offered an adequately coarse diet and cows must consume the coarse fractions of the diet. A cow's nose is about 5 inches wide; she can use it to push away feed particles that are a few inches long, especially on a dry diet. Some cows seem to sort extremely against these very long particles. Medium length fibers probably contribute to physically effective fiber and are much less likely to be sorted. Therefore, to insure an adequate intake of physically effective fiber by **all** cows in the herd, moist diets with intermediate particle size should be best. Intermediate particles are the finer particles on the top Penn State screen and some large percentage of the particles on the middle screen. One solution is well-managed hay crop silage.

### Introduction

This paper focuses on how we make sure that cows consume an adequate amount of physically effective fiber. For some people, the term physically effective fiber seems redundant, because to them, effective fiber means the same thing. Physically effective fiber is used to emphasize that adding fiber works in both a physical and chemical way. Coarse fiber

provides both important physical and chemical properties, and fine fiber provides only the chemical properties.

Physically effective fiber is essential to provide rumen fill and prevent abomasal displacement. Physically effective fiber forms a thick rumen mat that slows passage of smaller fibrous feed particles and increases their digestion. Finally, physically effective fiber enhances rumination and salivation and provides a better buffer source for the rumen. The latter is very important for cows eating large quantities of feed, much of which is rapidly fermented to organic acids in the rumen.

Nutritionists know that there are several diets on the farm: the one they told their clients to feed, the one actually being offered to the cows, and the one that the cow actually consumes. The last category also must include variations imposed by each cow on her own diet. This cow variation becomes an important factor in delivering the minimum amount of adequate physically effective fiber to each cow (or at least most cows) in the herd, which is different than offering a diet with adequate physical fiber and also different than having the average cow consume a diet with adequate physically effective fiber.

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## Basic Cow Behavior and Variation

Our first experiment on sorting (Leonardi and Armentano, 2003) sought to examine how cows vary in their sorting behavior and to explore other issues that we thought might be important. The design used 24 cows so that individual cow behavior could be measured. Six diets were fed in a Latin square design, and we measured sorting on several days within each of the six periods. Both of these features allowed us to distinguish cow effects from simple day-to-day variation.

Sorting was measured by determining the physical distribution of the feed refused by each individually housed cow. Actual as-fed intake of each screen (total offered – amount in refusals) was calculated directly. This number was divided by the predicted as-fed intake for that same screen, where predicted intake for a screen is the as-fed intake for that cow (total offered – total refused) multiplied by the as fed distribution of the total mixed ration (**TMR**) on that screen. So the predicted intake is the ‘value on paper’ for the diet and the numbers we show are the actual cow intakes of each screen, presented as a percentage of the predicted. When expressing data this way, screens with less than 100% are being sorted against and screens more than 100% are being selectively consumed. If one screen is less than 100%, some other screen will have to be more than 100%. In general, screens with a small amount on them (like the coarsest screen) can deviate from 100% more than screens with a lot of material on them, and it is important to remember this to avoid over-interpreting the data.

Figure 1 shows the behavior of the individual cows averaged across all six treatments (more about treatment effects will be discussed later). Note that the bottom line on Figure 1 represents the top screen from the 6-screen Wisconsin separator. In comparing this to the Penn State screens used in the field, remember that the top screen of the Penn State

shaker is roughly equivalent to the top two screens of the Wisconsin separator. Another way of saying this is that the longest particles that we have labeled as Y1 in Figure 1 are the coarser particles on the Penn State top screen, Y2 represents the finer particles on the Penn State top screen, and Y3 is roughly equivalent to the second (middle) Penn State box.

The most important thing to note from Figure 1 is that for those cows that did sort, they almost always sorted against the very longest particles, much less against the next coarsest particles, and selectively consumed the particles from the finest screen and pan. Although some cows (such as cow 3930 and 4178) showed very little sorting, and one cow (4454) showed reverse sorting, this trend is pretty strong. Also, there was a great deal of variation in how much individual cows sorted against the top screen. Cows 3894, 4156, 4454, and 4460 were repeat offenders, tending to sort more than the other cows across treatments.

## Forage Effects

Figure 2 shows the same data averaged across cows for each of the six treatments (20HQC through 40LQC). If you ignore what the treatments are for now, you can see more sorting occurred against the longest screens and the effect is rather linear, with sorting against long, no sorting in the middle, and preferential consumption of the finest material. The HQ and LQ refer to the quality of hay in the diet, where the lower quality hay (**LQ**) was 44.6% NDF and the higher quality hay (**HQ**) was 34.5% NDF. Diets contained 20% hay and 20% haylage (the diets marked 20), and the hay was either left long (HQL and LQL) or chopped (HQC or LQC) by overmixing in a horizontal auger TMR mixer. The 40HQC and 40HQL diets were 40% hay with no haylage.

The first surprise is that quality did not have an effect on sorting. We thought cows would be more likely to sort against lower quality

hay. It didn't happen. That doesn't mean that it will never happen, and it is important to note that the hay was not moldy or unsound (or even all that high in NDF). Also, when a low NDF hay is fed, NDF tends to be distributed throughout the screens somewhat more evenly, especially if non-forage sources of NDF are brought in to increase dietary NDF. In that case, even if sorting doesn't change, the effect of sorting on NDF intake might. But basically, we didn't see high hay quality as a solution to sorting.

You can see that the treatments 40HQC and 40LQC cause the most sorting. These are very dry diets (90% DM) with no silage, which most nutritionists have probably learned to avoid. Comparing the particle size distribution of the hay, chopped hay, and haylage used in these diets (Figure 3), we can see that hay chopped by processing for 15 minutes in a TMR mixer (middle set of bars) results in a bimodal or 'dumbbell' distribution with a lot of very long fibers but also a lot of fines. Haylage on the other hand, produces something more like a bell shaped curve with more particles on the Y2 and Y3 screens. The distribution and/or the DM content of the 40% chopped hay diets (40HQC and 40LQC) made these diets very sortable. The mixed rations for these dietary treatments (Figure 4) had roughly 15% on the Y1 and Y2 screens combined, but the chopped hay diets had more fines than the diets with haylage and less material on the middle screens.

What happens when we take a sortable diet like these last two and combine them with a picky cow (like cow 4454) can be seen in Figure 5. The treatment effects of Figure 2 are greatly exaggerated in this cow. Look closely at the last set of bars and you will see that this cow ate none of the longest particles on diet 40LQC (40% lower quality chopped hay with no haylage). This was based on observations of two consecutive days, starting five days after first offering the diet. Its possible that she didn't sort as badly on other days, and she certainly didn't

sort any worse. But, this cow was obviously getting no physically effective fiber from the longest particles on these two days. In fact, this extreme sorting reduced the mean particle size from 0.13 inches (3.35 mm) to 0.09 inches (2.26 mm), or a reduction of roughly one third. Later, we'll show how different particle size distributions can be affected differently when assigning similar levels of sorting on each screen.

### Importance of 'Top' versus 'Middle' Screens

Recommendations generally call for a minimum percentage (7 to 8% is a common recommendation) of a TMR on the top Penn State screen. This is obtained by using forages with even higher percentage on the top screen and mixers that don't reduce this value much during mixing. Its important to realize that the Penn State screens don't really allow calculating mean particle size, and even the cumbersome Wisconsin screens only 'estimate' particle size. Also, we rarely account for DM and never fiber content on the screens. In general, within a forage type, the mean particle size is going to be highly correlated with the fraction on the top Penn State screen. For example, a coarse haylage will have more on the top screen and less on the pan. However, as in the case of chopped hay versus haylage or long hay in Figure 3, this correlation may not hold well across different forage types. Therefore, although we make recommendations based on percentage on the top screen, it may well be that a similar mean particle size obtained by a large percentage on the middle screens may perform similarly to the same mean particle size achieved with more longer particles. What may be different is that the latter diet may be more susceptible to sorting, and therefore, it will not result in delivery to the cows rumen what appeared on paper.

We conducted an experiment using oatlage processed to achieve different particle lengths (Leonardi et al., 2001). The goal was to obtain two TMR with equal mean particle sizes



but with one diet getting more of its mean particle size from very long particles and the other from more intermediate fibers. How we did this is shown in Figure 6. Both silages were processed in the field to provide long or medium particle length. Then, the long oat silage was finely chopped just prior to feeding. This provided three particle lengths for diets that we named long oat silage (**LOS**), medium oat silage (**MOS**), and fine from long oat silage (**FLOS**). We also made a fine silage daily from the medium oat silage (**FMOS**) to compare to FLOS. The last comparison was just to make sure there weren't any effects due to ensiling (and there weren't). The last, and most important treatment, was obtained by mixing long oat silage with chopped oat silage to make a mixed oat silage. The TMR with this oat silage was called LFLOS to represent a mixture of long oat silage and fine (from long) oat silage. The important comparison is between the MOS and the mixed fine and long oat silage diets.

The mean particle size of the diets and their particle distribution are shown in Figure 7. Note that the MOS and LFLOS diets have about the same mean particle size in the diets offered (5.19 and 5.39 mm, respectively). Also, note that the LFLOS mixed diet has more long particles (Y1 and Y2) and less medium particles (Y3 and Y4) compared to the medium MOS diet. In Figure 8, rumination is plotted against mean particle size of the diets as consumed (that is, after taking into account each cow's sorting). Mean particle size does a rather good job of predicting rumination, an indicator of physically effective fiber content of the diet. Although not shown, the percentage of feed on the top two screens also would be an indicator of physically effective fiber. My interpretation of this trial is that the medium particles probably do contribute to the physically effective fiber in the diet, but this is based more on the inability to show something special about the long fibers.

## Extrapolating From Our Observations

If the assumption that mean particle size is a good estimator of the physical effectiveness of the diet is true, two things must follow. First, the value of material on the top and middle screens toward physical effectiveness is simply a function of how they affect mean particle size, something that we can estimate mathematically, at least for well characterized particle size distributions. Secondly, we can also estimate the impact of sorting on different types of diets to estimate the mean particle size consumed.

Based on their contribution to mean particle size, it is possible to roughly estimate that 5% on the Y1 screen is equal to 7% on the Y2 screen or 10% on the Y3 screen. In this calculation, the 'excess' was coming from the Y5 screen. So, one way to look at this is that it takes about twice as much increase in the middle screen to offset a decrease in the coarsest particles in the as-fed diets. But, what if a cow eats most of the middle screen particles and extensively sorts the very coarse particles? Is it possible that, at least for the extreme sorting cows, that the diets with mean particle size coming from the middle screen will actually deliver more physically effective fiber to the cow than a diet with similar mean particle size coming from long fibers?

We did this calculation on two 'imaginary' diets shown in Figure 9. Note that TMR 2 has 6.6% on Y1 versus 0% for TMR 1, but TMR 1 compensates by having 28% on Y3 versus 15% for TMR 2 (a difference of 13%). We applied sorting coefficients of the cow eating 60% of Y1 offered, 80% of Y2, and 90% of Y3. For TMR 1, this was compensated for by 105% consumption of Y5 and 110% consumption of Pan and for TMR 2 by 108% consumption of Y5 and 115% consumption of pan. It is easy to see that similar sorting has a larger effect on TMR 2, reducing the mean particle size from 4.35 to 3.72 mm (about 15%) compared to 3.99 for TMR 1 (8% reduction). Also, notice that

the change in mean particle size is fairly large if you compare it to the wide range achieved in the oat silage trial shown in Figure 8. Clearly, by looking at the degree of sorting expressed by some cows on diets very susceptible to sorting, the changes could be much more extreme.

Note that we applied the same sorting to these two diets. Sorting in the oatlage trial is shown in Figure 10. Sorting was significantly increased as mean particle size increased. Obviously, increasing mean particle size offered will still increase mean particle size consumed, just not as much as the paper diets tell you. There was also a significant difference in the pattern of sorting caused by the particle size distribution.

### Addition of Water

It is a common practice to add water to dry diets fed to dairy cattle. Our first experiment compared hay to haylage to achieve different particle size distributions, and changes in sorting could be attributed to either distribution or DM. In the oatlage trial (Leonardi et al., 2001) there was some evidence that distribution had an effect and moisture was not a factor in this experiment as all diets were about 48% DM. What about the effect of moisture, independent of particle size distribution? We examined this by using chopped hay in diets with and without added water (Leonardi et al., 2002). Realize that when water is added to diets, feeds will cling together, increasing the apparent particle length of the diets. We wouldn't expect this artificial increase in mean particle size to have any effect on the physical effectiveness of the diet offered; however, it could affect sorting. In these trials, we measured sorting using the same as-fed shaking apparatus as before, but after shaking, we determined the DM. We did not use wet sieving of the diets but that probably would have been a better approach to determine true mean particle size.

Dry matter content of the diet was reduced from 80.2% to 64.3% by adding water.

There was some statistically significant reduction in sorting due to water addition (Figure 11). We added as much water as we could without making the rations sloppy, and presumably on the farm, much less water would be added. Perhaps even more importantly, when individual cow sorting was measured, both the dry diets and the wet diets had some cows that sorted extremely against the longest particles on two consecutive days (Figure 12). It is interesting that they weren't the same cows in this switchover trial. The net result is that water addition was slightly helpful in reducing sorting, but some cows still sort strongly against the coarsest particles.

### Summary

The most important observations to remember are that most of the impact on sorting is on the coarsest particles or the top Y1 screen of the Wisconsin separator. This screen correlates to the coarsest particles on the Penn State top screen. The Y2 and Y3 screens are much less likely to be sorted against and therefore deliver somewhat less physically effective fiber but to a higher percentage of cows.

Using dry hay or straw processed to provide very long particles can increase the mean particle size of the diet with a small amount of DM. However, the use of this long fiber is more likely to result in the diet on paper underestimating the mean particle size of the diet consumed, especially for some cows. The dry nature of these materials probably makes this even worse, and adding water does not cure the problem.

No one has yet established whether cows that sort excessively against long fibers are more prone to the physiological problems associated with offering diets low in physically effective fiber. Also, we are not aware of evidence to show that providing adequate mean particle size from large amounts of medium sized fibers is better than providing the same particle length from a

‘bimodal’ mixture of short and long fiber. The recommendations that follow are what we perceive as common sense interpretations of the data that we have with dietary effects on sorting, on individual cow behavior, and on the clear tendency for some cows to sort worst against the very long fibers.

Making sure that a cow is offered adequate amounts of physically effective fiber is not rocket science. This can usually be done by simple good management practices when harvesting and ensiling silage and haylage and by avoiding overmixing of diets. This seems to be the cheapest and most reliable way to promote adequate consumption of physically effective fiber by dairy cows. Adding coarsely chopped or long hay is another common way of adding physically effective fiber. While this certainly works on the diet offered, and your Penn state box numbers will look good on paper, it will only work on the cow that uniformly eats the diet.

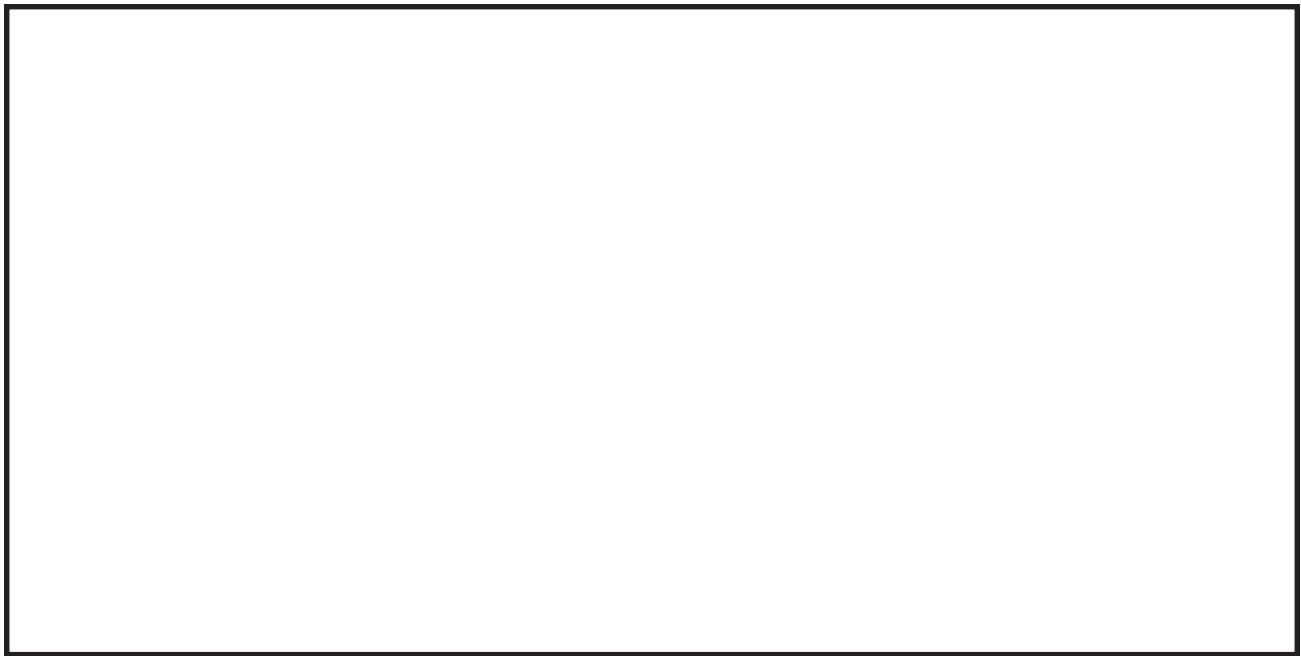
To paraphrase Abe Lincoln, you can get some of the cows to eat all their long particles some of the time, but you cannot get all of the cows to eat all of the long particles all of the time. In other words, some cows sort. We are less concerned about the average amount of sorting in the herd being fed diets with normal moisture than we are about the extreme sorting that can be done by a few cows. Adding very long fiber to the diet (or free choice feeding of long hay) raises the average physical fiber intake of the herd, but is adding this ‘avoidable’ fiber the best way to protect cows that are extremely picky? Probably not.

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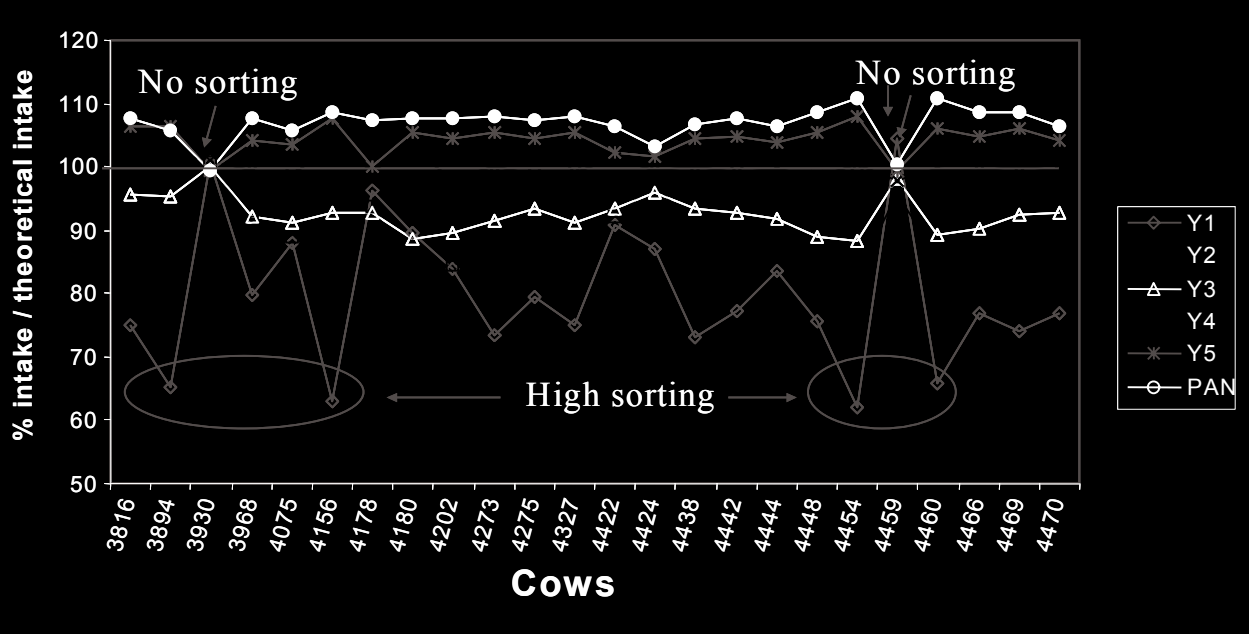
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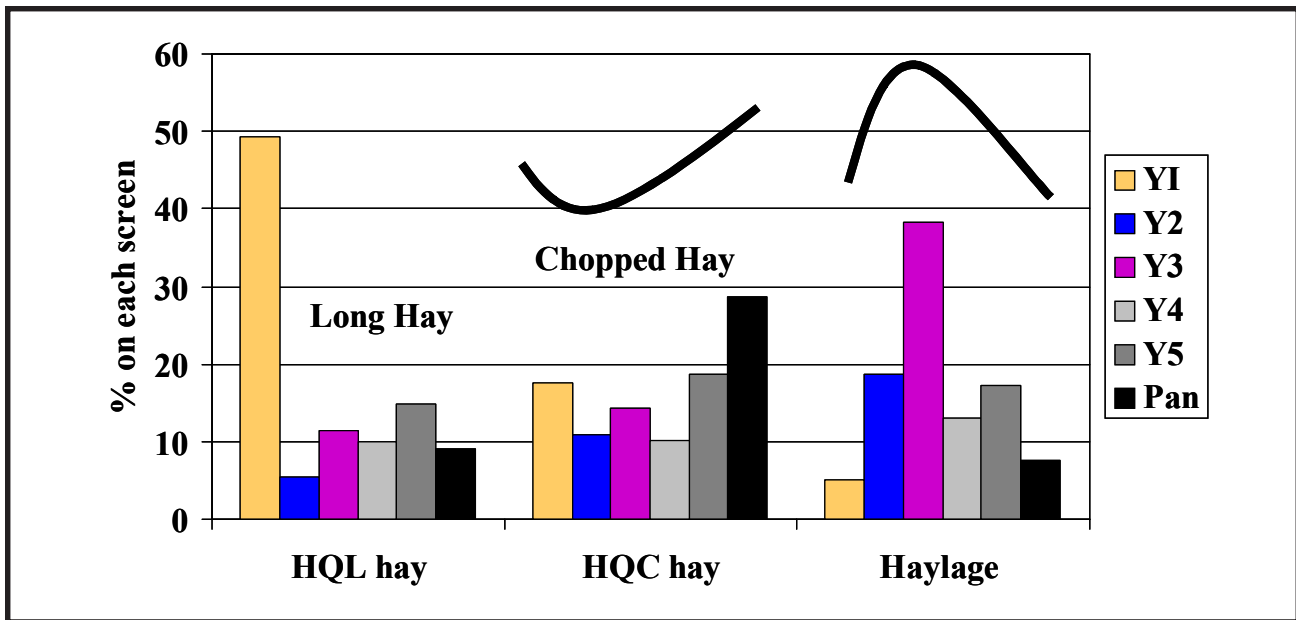
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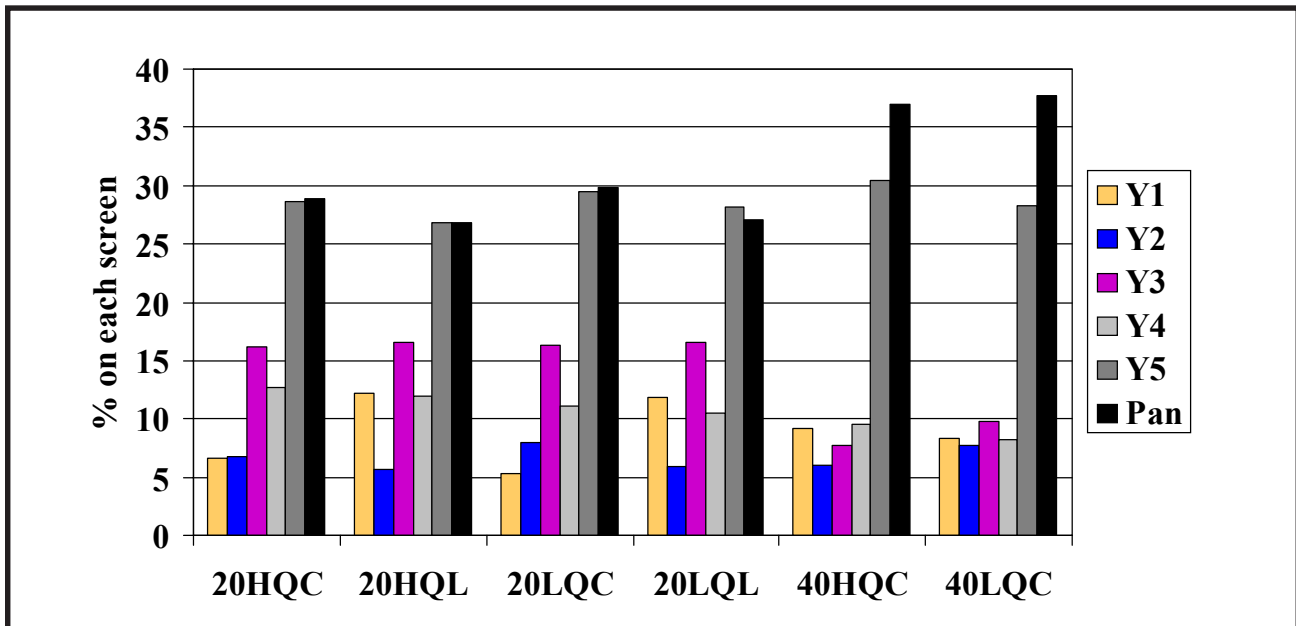


**Figure 1.** Sorting of different sized particles (Y1 = longest, Pan = shortest) by individual cows. Numbers less than 100% indicate particles are sorted against, numbers greater than 100% means that particles are preferentially consumed. These data points were averaged across 12 days, 2 days each from six different treatments. Reprinted from Leonardi and Armentano, 2003.

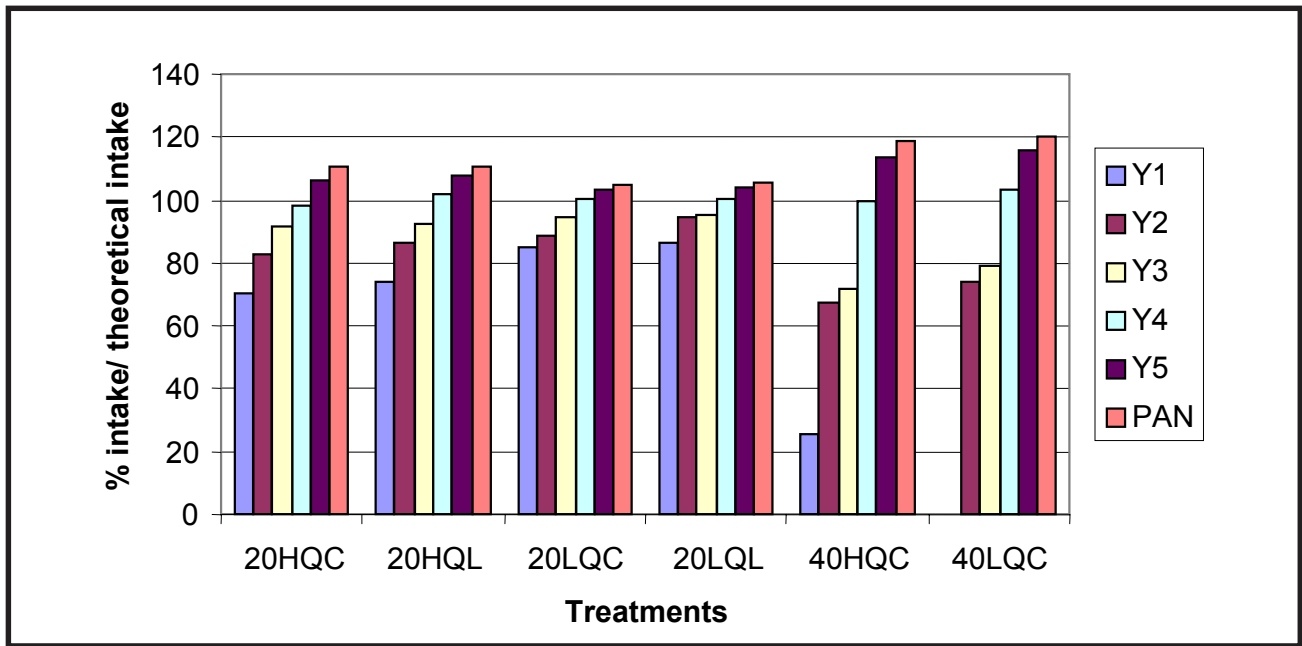




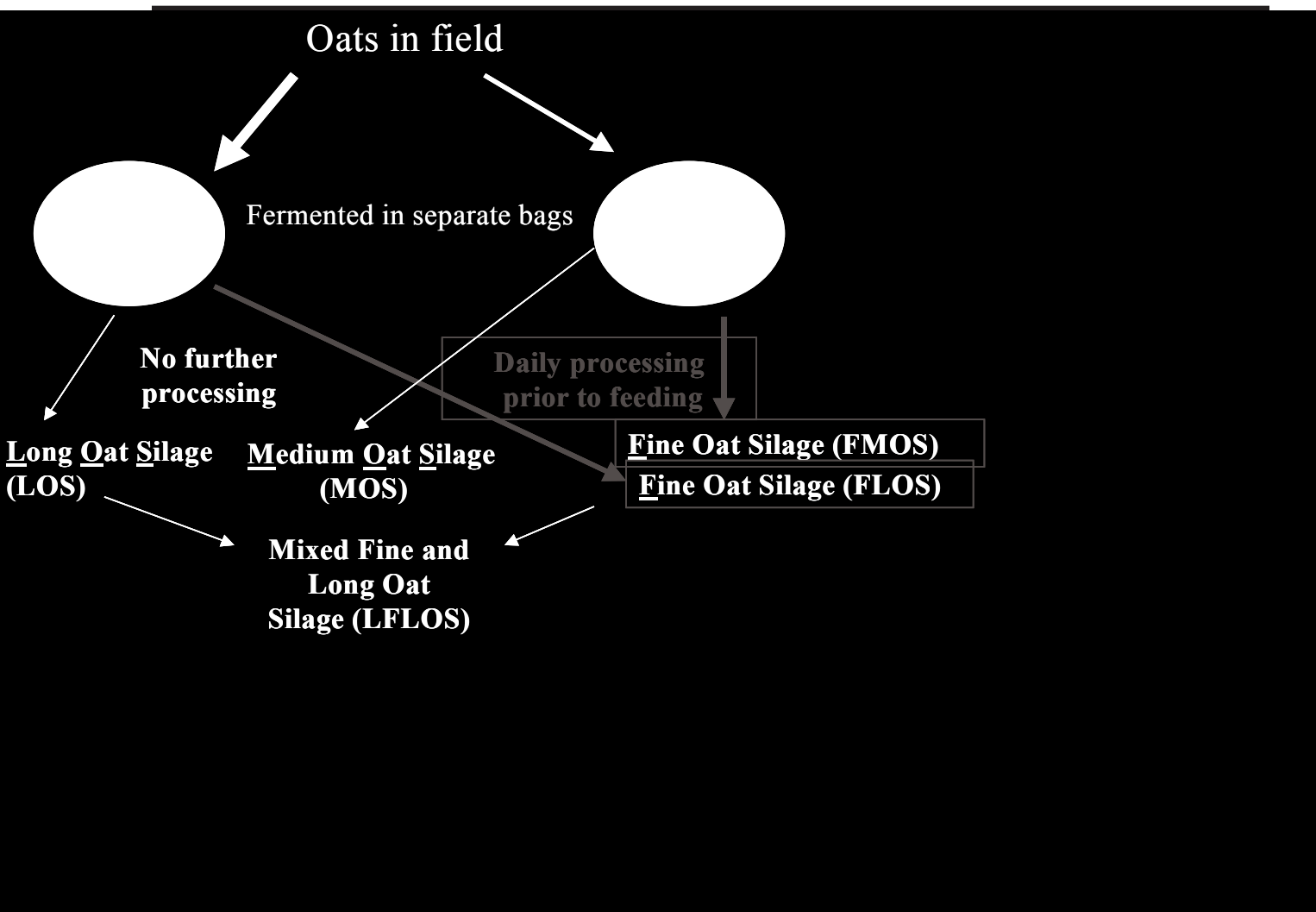
**Figure 3.** Particle size distribution of high quality long hay (HQL), high quality chopped hay (HQC) and haylage. Particle size shown from longest (Y1) to shortest (Pan). Taken from Leonardi and Armentano, 2003.

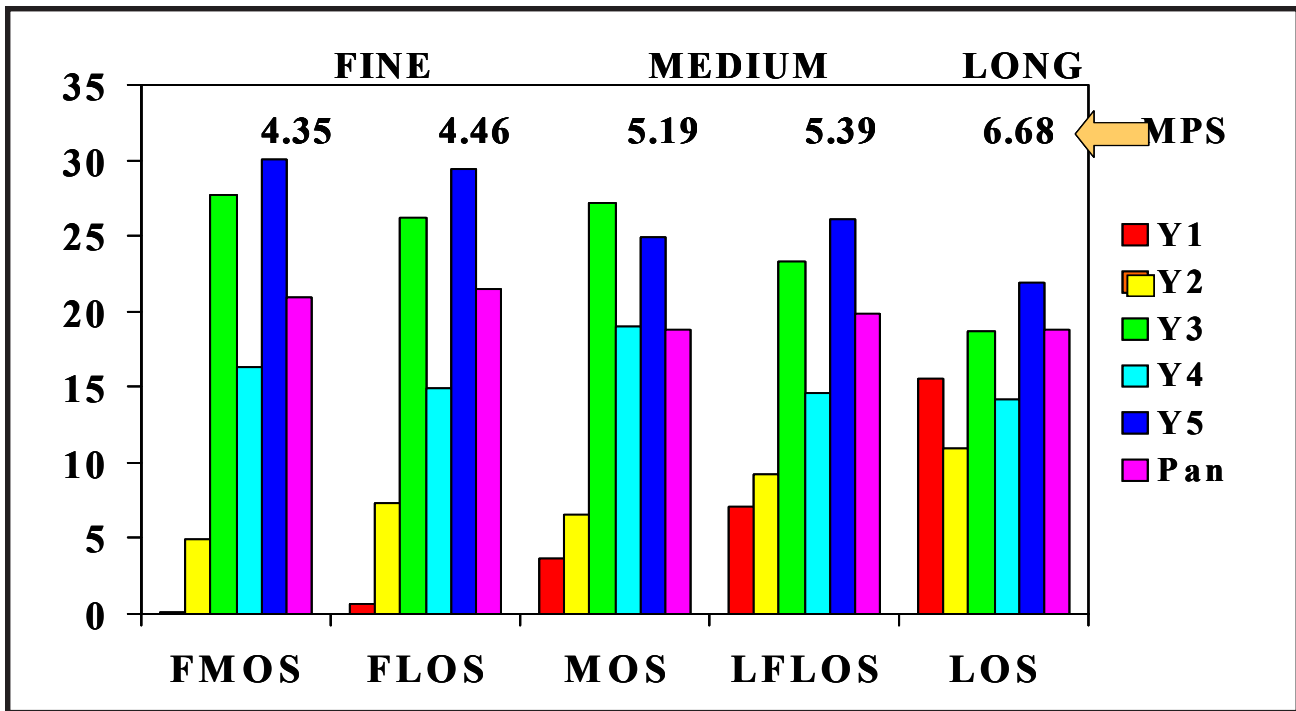


**Figure 4.** Particle size distribution of total mixed ratios from longest (Y1) to shortest (Pan). Treatments are 20HQC = 20% high quality chopped hay, 20 HQL = 2-% high quality long hay, 20LQC = 20% low quality chopped hay, 20LQL=20% low quality long hay, 40HQC=40% high quality chopped hay, and 40LQC=40% low quality chopped hay. Reprinted from Leonardi and Armentano, 2003.

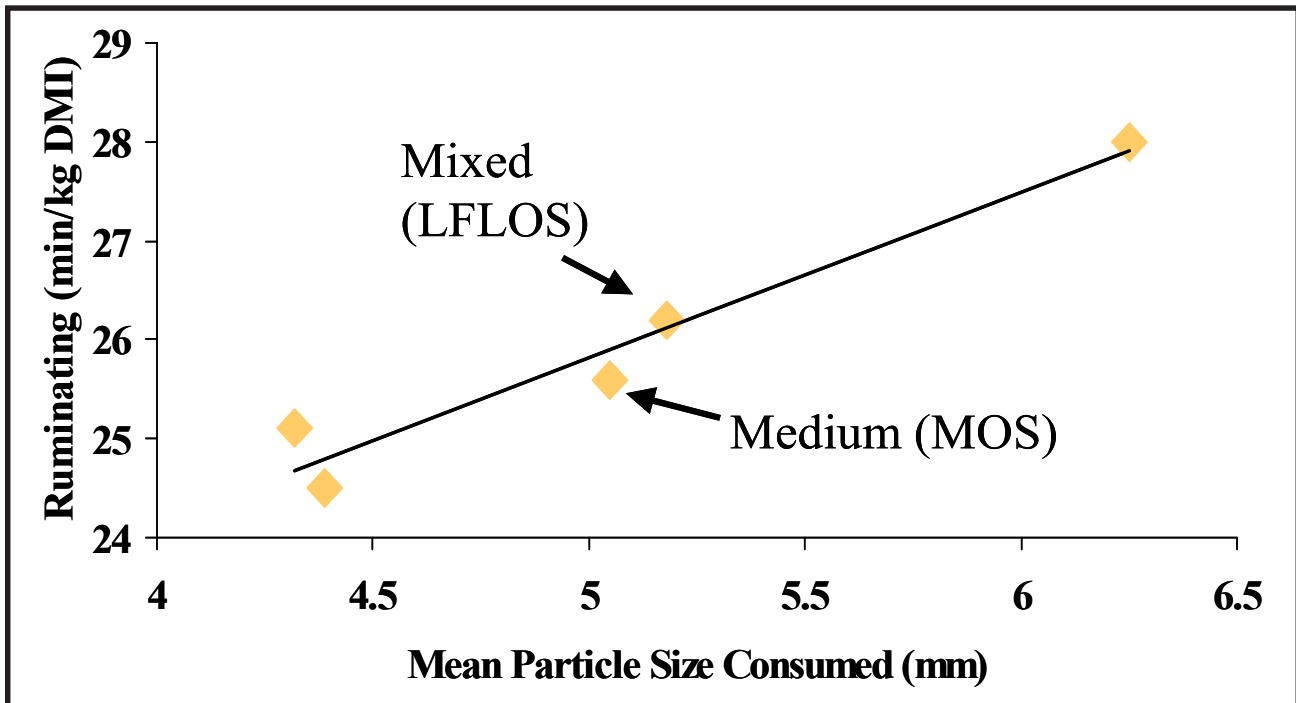


**Figure 5.** Sorting by a single cow (4454) of the six diets. Data represents sorting on two consecutive days. Treatments are 20HQC = 20% high quality chopped hay, 20HQL = 2-% high quality long hay, 20LQC = 2-% low quality chopped hay, 20LQL = 20% low quality long hay, 40HQC = 40% high quality chopped hay, and 40LQC = 40% low quality chopped hay. Taken from Leonardi and Armentano, 2003.

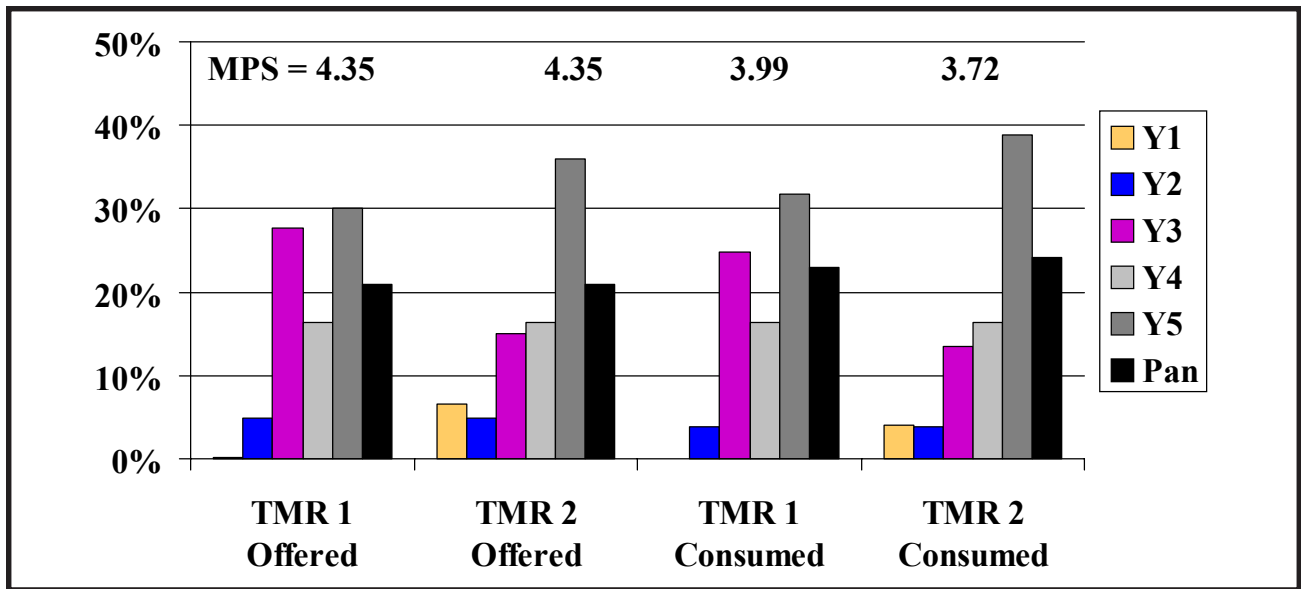




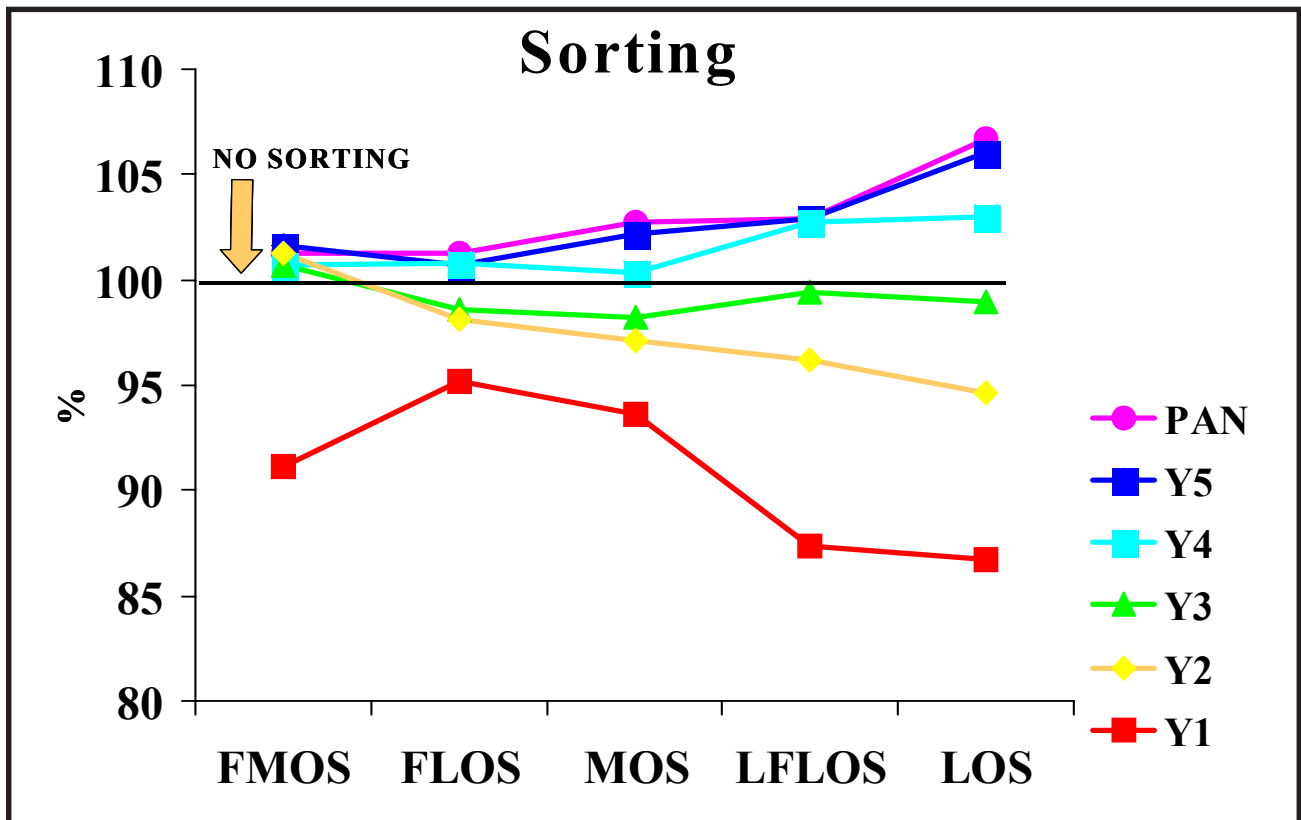
**Figure 7.** Particle size distribution and mean particle size (MPS, mm) of total mixed rations (Leonardi et al., 2001). FMOS = fine from medium ensiled oat silage, FLOS = fine from long ensiled oat silage, MOS = medium ensiled oat silage, LFLOS = mixed long and fine from long ensiled oat silage mixed to give particle size similar to MOS, and LOS = long ensiled oat silage.



**Figure 8.** Relationship of ruminating time to mean particle size of diets consumed (Leonardi et al., 2001). Particle size of diets has been adjusted to reflect sorting. LFLOS = long from fine plus long oat silage, and MOS = medium oat silage. Rumination is proportional to mean particle size ( $P < 0.01$ ) and no difference exists between medium (MOS) or mixed (LFLOS,  $P = 0.46$ ).

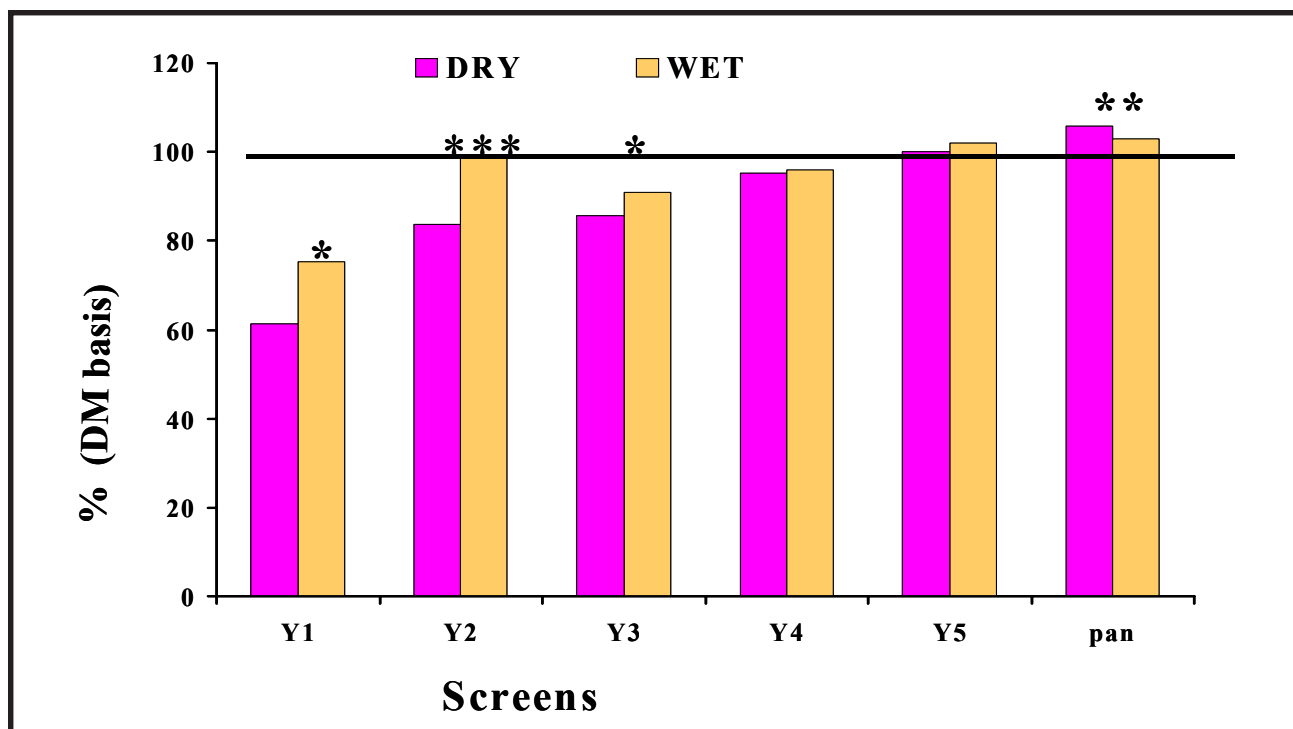


**Figure 9.** Effect of sorting of two different total mixed rations (TMR) with equal as fed mean particle size (MPS). Sorting was consuming 60% of top screen (Y1), 80% of second screen (Y2), 90% of screen Y3, 100% of screen Y4, 105 to 108% of screen Y5, and 110 to 115% of pan.



**Figure 10.** Sorting in the oat silage trial (Leonardi et al., 2001). Y1=longest particles through pan=shortest. FMOS = fine from medium ensiled oat silage, FLS = fine from long ensiled oat silage, MOS = medium ensiled oat silage, LFLOS=mixed long and fine from long ensiled oat silage mixed to give particle size similar to MOS, and LOS = long ensiled oat silage.



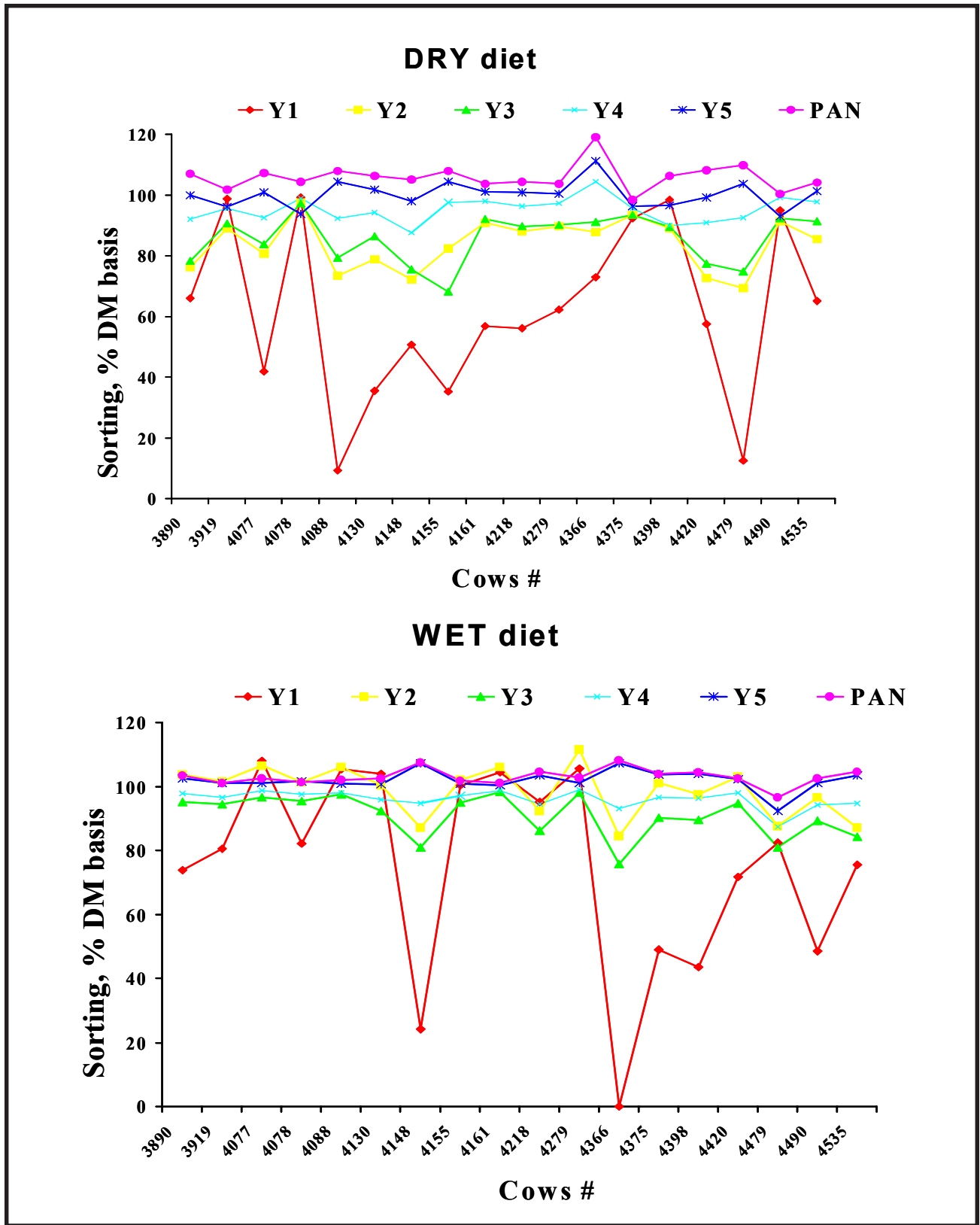


**Figure 11.** Effect of adding water on sorting of particles from longest (Y1) to shortest (pan) (Leonardi et al., 2002).

\* =  $P < 0.05$ .

\*\* =  $P < 0.01$ .

\*\*\* =  $P < 0.001$ .



**Figure 12.** Sorting by individual cows when fed the wet or dry diets (Leonardi et al., 2002). Y1 = longest particles through pan = shortest.



## Forage Alternatives

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### Abstract

Forage alternatives can be used to reduce the filling effects of diets and to stretch available forages when their supply or quality is limited. Dietary space vacated by forage is filled primarily by high fiber byproduct feeds or by cereal grains; the extent to which they can substitute for forage depends primarily on their digestion characteristics - they should be slowly fermented with high total tract digestibility. Diet fermentability must be limited to prevent excess ruminal acid production. Adequate coarse fiber particles should be included in the diet for formation of the rumen mat to entrap and retain small potentially fermentable fiber particles, increasing their digestibility.

### Introduction

Forage availability is sometimes limited because of poor growing conditions or because of insufficient land base on individual farms. In addition, forage fiber can limit feed intake, particularly for high producing cows, and forage quality is highly variable. Therefore there is continual interest in alternatives to forages in diets for dairy cows. Decreasing the forage content of diets requires careful selection of ingredients for use in the dietary space created. Because cereal grains must be limited in diets to avoid ruminal acidosis, byproduct feeds with high fiber content are the most common alternatives to forages and can be used to replace

some, but not all, forage in diets of lactating cows. These byproduct feeds are referred to as non-forage fiber sources (**NFFS**) to distinguish them from other byproduct feeds. However, cereal grains can also be used in place of forages to some extent if fermentability of starch is reduced. Forage alternatives should be considered based on their ability to functionally substitute for forage. This paper discusses how forages function in diets of dairy cows and factors to consider when selecting forage alternatives.

### Forage function

Forages are unique compared to other dietary ingredients because they provide long fibrous particles that are retained in the rumen longer, and tend to ferment more slowly, than smaller feed particles. This provides a consistent source of fuels to microbes in the rumen, as well as a basal supply of fuels to the liver and mammary gland over time, allowing greater milk yield. Some long fibrous particles are necessary for formation of the rumen mat, which entraps small particles, increasing their ruminal digestibility. An adequate mass of digesta in the rumen is required to stimulate chewing, which increases secretion of salivary buffers.

A high basal fuel supply from fiber fermentation in the rumen is highly desirable and dependent on the digestion characteristics of the fiber source. Forage fiber should have a high

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turnover rate from both digestion and passage to maximize fuel supply, while minimizing the filling effect of the fiber over time. Increased passage of particulate matter is also expected to increase efficiency of microbial protein production by increasing passage of attached microbes before lysis occurs. However, forages vary considerably in their digestion characteristics, and it is often desirable to find alternatives for forages if their quality is poor.

Forages also dilute rapidly fermentable feeds, such as cereal grains in diets, thus preventing excessive ruminal fermentation. However, this function is not unique to forages, and the ability of feeds to slow ruminal fermentation while providing high total tract digestibility determines their value as a forage alternative. The concentration of forage in diets can often be decreased while maintaining or enhancing feed intake, milk yield, and health. The goal is to provide cows low-fill, highly fermentable diets that result in consistent fermentation over time.

### **Filling dietary space vacated by forages**

#### *Non-forage fiber sources*

The primary advantage of NFFS is that they generally have higher energy concentrations and are much less filling than forages; feed intake is limited to a greater extent when the neutral detergent fiber (**NDF**) concentration of the diet increases from forage compared to NFFS (Allen, 2000). They are commonly used as forage alternatives and vary widely in cost, availability, and chemical composition across type and location, and over time. Compared to cereal grains, NFFS are less likely to result in ruminal acidosis because they generally ferment more slowly, are not fermented to lactic acid, and because their rate of fermentation slows as ruminal pH declines. Like forages, NFFS often result in decreased propionate and increased acetate production in the rumen when substituted for cereal grains (Harvatine et al., 2002;

Ipharraguerre et al., 2002; Voelker and Allen, 2002). A slower rate of fermentation and decreased propionate production might allow increased meal size and greater feed intake when they are limited by absorbed propionate (Allen, 2000). However, substitution of NFFS for forage usually decreases the ratio of acetate to propionate concentrations in the rumen (Weidner and Grant, 1994; Clark and Armentano, 1997; Mowrey et al., 1999), as well as ruminal pH (Weidner and Grant, 1994; Harvatine et al., 2002). Wisconsin researchers reported that NFFS were only about one half as effective as alfalfa NDF at elevating milk fat concentration and were less effective at stimulating chewing activity when substituted for grain in low forage diets (Swain and Armentano, 1994). Another Wisconsin experiment found that NFFS were only 27% as effective at increasing milk fat concentration as alfalfa silage NDF (Pereira et al., 1999). Therefore, when replacing forage with NFFS, all non-forage components must be considered for their effects on diet fermentability.

The fiber concentration (NDF and soluble fiber) of most NFFS are in the range observed for most forages (40 to 60% on a DM basis), but some NFFS have NDF concentrations exceeding 75% (oat hulls, cottonseed hulls, and ground corn cobs). The primary difference in the fiber of NFFS compared to forages is that particle size of NFFS is smaller, so they cannot provide long fibrous particles to form a rumen mat. While some NFFS, such as cottonseed hulls and oat hulls, are poor sources of energy and protein and function only to dilute rapidly fermentable grains, most NFFS are good sources of fermentable fiber. Some have additional value because they provide fatty acids and(or) ruminally undegraded protein. Soyhulls and beet pulp are excellent sources of fermentable fiber but have low protein and fatty acid concentrations. Crude protein concentrations of distiller's grains, brewer's grains, corn gluten feed, and whole linted cottonseeds are higher than for most forages, ranging from 24 to 30% of DM. Whole cottonseeds, and to a lesser extent

distiller's grains, provide additional energy as fatty acids. The extent to which each NFFS should be used as a forage alternative depends on its availability and price relative to other energy and protein sources, as well as its ability to substitute functionally for forage.

Recent research with lactating cows suggests that NFFS not only reduce the amount of starch fermented in the rumen when substituted for grain but also reduce the ruminal digestibility of the remaining starch. Illinois researchers substituted soyhulls for dry ground corn at 0, 10, 20, 30, and 40% of dietary DM and reported that ruminal digestibility of non-structural carbohydrates tended to decrease linearly from nearly 30% to less than 5% without reducing ruminal or total tract digestibility of organic matter (Ipharraguerre et al., 2002). We recently reported similar effects on ruminal starch digestibility when pelleted beet pulp was substituted for high moisture corn at 0, 6, 12, and 24% of dietary DM (Voelker and Allen, 2002). The amount of starch truly digested in the rumen decreased dramatically from 8.4 to 1.5 lb/day, partly because of the expected reduction in starch intake from lower dietary starch concentration but also from an unexpected reduction in true ruminal starch digestibility from 47 to 17% as beet pulp replaced high moisture corn in diets. There was no effect of treatment on total tract starch digestibility, despite this large reduction in ruminal starch digestion because of compensatory postruminal starch digestion. In addition, true digestibility of organic matter in the rumen was not affected by treatment because of increased NDF digestibility; this increase resulted in increased apparent total tract digestibility of organic matter as beet pulp replaced corn in the diet. The dramatic reduction in ruminal starch digestibility was because rate of ruminal starch digestion decreased from 11.3 to 1.7%/h and rate of starch passage from the rumen increased from 15.9 to 23.5 %/h. It is not known if other NFFS have the same effects on rate of digestion and passage from the rumen, so this should be investigated

further because it has important implications for limiting ruminal starch digestion. It might not be desirable to reduce ruminal starch digestibility to the extent observed with the highest substitution rates of beet pulp or soyhulls, but the linear responses obtained in these two experiments suggest that NFFS can be used at lower substitution rates to manipulate ruminal starch fermentability and site of starch digestion. Therefore, substitution of NFFS for both forage and grain might be necessary to limit diet fermentability as the forage content of the diet decreases.

A limitation of NFFS as a forage alternative is that particle length is not adequate for formation of the rumen mat. In addition, passage rate is generally greater for NFFS NDF than for forage NDF (Firkins, 1997), and ruminal digestibility can decrease if poor mat development reduces their retention time in the rumen. If adequate long particles are provided by coarse forage in the diet, NFFS can contribute to mat formation because they tend to be buoyant and become entrapped. Another limitation of NFFS is that, compared to cereal grains, NFFS provide fewer glucogenic precursors, which can be limiting for milk production, particularly for high producing cows.

### *Cereal grains*

Although one doesn't normally think of cereal grains as forage alternatives, they can also be used in the dietary space vacated by forage. Cereal grains contribute less to digesta mass in the rumen than NFFS because their retention time and water holding capacity are lower. The extent to which a cereal grain can replace forage in the diet without reducing feed intake or causing ruminal acidosis depends on its digestion characteristics. Cereal grains with moderate ruminal fermentability and high whole-tract digestibility, such as dry ground corn, are desirable to include in diets in place of forage. They are less filling than forages and have higher energy concentrations. They have

an advantage over NFFS because more glucogenic precursors are provided from starch digestion compared to fiber digestion. Highly fermentable starch sources, such as finely ground high moisture corn, barley, wheat, and bakery waste, should be avoided to prevent excessive fermentation. Starch sources with low total tract starch digestibility, such as unprocessed sorghum or coarsely cracked corn, should also be avoided because they reduce dietary energy density.

Reduced ruminal fermentability and propionate production might result in increased meal size and feed intake. A recent experiment from our laboratory showed that meal size increased from 4.2 to 5.1 lb and feed intake increased from 45.8 to 49.5 lb/day, as dry ground corn replaced high moisture corn in high starch diets fed to lactating dairy cows (Oba and Allen, 2003a). True ruminal starch digestibility decreased from 71% for high moisture corn to 47% for dry ground corn with no effect of treatment on total tract starch digestibility (95%; Oba and Allen, 2003b). Although milk yield was similar for the two treatments, fat-corrected milk yield tended to be 6.6 lb/day higher for dry ground corn compared to high moisture corn treatment because of higher milk fat concentration. When forage is replaced with cereal grains, those grains with slower ruminal fermentation are likely to improve feed intake and milk production, depending on their digestion characteristics and other dietary characteristics as discussed below.

### **Considerations for feeding low-forage diets**

The forage concentration of diets can be reduced by forage alternatives in many situations; in some, forage alternatives can replace a large fraction of the forage, allowing very low forage diets to be fed. Ohio researchers suggested that diets with forage NDF contents as low as 9 to 11% with whole cottonseeds and 14 to 16% without whole cottonseeds can be fed to mid-lactation cows when non-structural carbohydrates are limited to 30% of DM (Slater

et al., 2000). Nebraska researchers reported that a wet corn milling product (40% NDF, 23% crude protein) has the potential to replace all of the concentrate and up to 45% of the forage in the diet for lactating cows (Boddugari et al., 2001). The forage content of diets can be minimized by considering the following recommendations.

#### *Include forages with high fiber concentrations and long particles*

Forages vary greatly in fiber concentration and particle size, and long fibrous particles are what distinguish them from other feed ingredients. Therefore, the forage content of diets can be reduced if forage with higher fiber concentrations and longer particles are used. For instance, a diet formulated using a forage with 50% NDF can contain 20% less forage to provide the same forage NDF concentration compared to a forage with 40% NDF, because more fiber is provided per unit of forage weight. However, it is also important to consider digestibility of forage NDF; increasing forage NDF by delaying harvest will result in decreased rate and extent of NDF digestion, which will decrease the basal energy supply to microbes and to the animal. Across cuttings and maturities, NDF concentration is poorly related to digestion characteristics of NDF for alfalfa (Allen and Oba, 1996), allowing opportunity to purchase or select forage with both high NDF content and high NDF digestibility. Corn hybrids with high NDF and high NDF digestibility exist and can be selected when land is limited for forage production. Forages can be compared for NDF digestibility by *in vitro* rumen fermentation or by evaluating the lignin concentration as a percentage of NDF; within a forage type, digestibility of NDF decreases as lignification of NDF increases (Allen, 2001).

Less forage is also needed in the diet if it is long or coarsely chopped. Fibrous particles of finely chopped alfalfa or corn silage are not as effective at forming a rumen mat as are longer

particles from coarsely chopped forage. Addition of coarse fiber as chopped hay improved digestion of soyhulls in several experiments (Grant, 1997); chopped hay increased rumen mat consistency and rumination time and decreased passage rate of wet corn gluten feed in another experiment (Allen and Grant, 2000). The length and concentration of long particles required in diets is not constant and depends on their digestion characteristics, as well as characteristics of other dietary ingredients.

#### *Avoid rapidly fermented feeds*

Rapidly fermented feeds containing sugars and starch should be limited. It is very important to consider the fermentability of *all* dietary ingredients when feeding low forage diets because most forage alternatives are more fermentable than forages and are also less effective at stimulating chewing. Limiting rapidly fermented feeds, such as finely ground high moisture corn and molasses, will reduce the risk of acidosis.

#### *Manage to avoid slug feeding*

Diets with less coarse fiber require less chewing during eating and can be consumed more quickly, resulting in larger meals before satiety occurs. Although this might be desirable to increase feed intake in some situations, it can also result in ruminal acidosis if fermentation acids cannot be absorbed quickly enough and the buffering capacity of ruminal contents is greatly exceeded. Overcrowding and feeding to an empty bunk increases competition among animals and encourages slug-feeding and should be avoided when feeding minimum forage diets.

#### *Include dietary buffers*

Although efforts should be made to reduce fermentability by limiting highly fermentable feeds and to reduce slug-feeding by providing adequate bunk space, inclusion of

buffers makes sense as another way to reduce the risk of acidosis.

#### *Limit NFFS with high fat content*

Concentration and availability of polyunsaturated fatty acids might limit inclusion rates for some NFFS, such as whole cottonseeds and distiller's grain, because of potential milk fat depression from the production of trans fatty acids by ruminal fermentation. Limitations depend on fatty acid concentration and composition of the NFFS and other dietary ingredients.

### **Conclusion**

Numerous strategies exist to reduce dietary forage content. Some coarse forage is needed in diets for retention of small particles in the rumen, increasing their digestibility. When replacing forage with other feeds, it is important to consider digestion characteristics of not only the forage alternative but of all dietary components.

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## Nutritional Considerations for Jersey Cows

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### Abstract

There are numerous suggestions in the popular press that the nutritional requirements and management of Jersey cows can not be based solely on recommendations for Holstein cows. However, in many cases, these recommendations are based more on anecdotal evidence and practical experience than on results of studies or data integrations designed to test breed differences. Many of these issues need to be clarified. For example, the high milk fat content of the Jersey is often the basis of suggestions that Jersey cows have a higher fiber requirement than Holstein cows, but other recommendations suggest that Jersey cows can tolerate higher dietary starch levels than Holstein cows and should be fed less fiber to achieve maximal intake. The Jersey is often purported to have a higher dry matter (**DM**) intake capacity per kilogram of body weight (**BW**) than the Holstein, but in many cases, this is related to differences in milk energy output per kilogram of BW or  $BW^{0.75}$ , which will increase appetite. However, there are data suggesting a higher rate of passage for the Jersey, which could allow greater intake, as well as affecting starch and NDF digestion. It is generally accepted that Jersey cows are more prone to milk fever, perhaps due to difference in vitamin D receptors; therefore, it has been suggested that their target dietary cation:anion difference be lower during transition. There are numerous reports in the literature that dry Jersey cattle have a higher maintenance energy

requirement than Holstein cattle, but in a calorimetry trial at Beltsville, MD, there was little difference in the energy metabolism of lactating Jersey and Holstein cows when the data were expressed on a  $BW^{0.75}$  basis. There was a tendency for the Jersey cows to partition more metabolizable energy towards milk than body tissue, but on a  $BW^{0.75}$  basis, the incremental use of metabolizable energy for net energy was remarkably similar for the two breeds.

### Introduction

A brief perusal of web sites and the popular press articles provides immediate evidence the Jersey breed has a passionate and enthusiastic following. Numerous surveys of herd records and data bases suggest a long list of advantages compared to other breeds, such as easier calving, reduced mastitis and lameness, and greater longevity. They are purported to have a good 'temperament' and be easy to handle, be 'easy' on pastures (they weigh less) and need less acreage, and are believed to be more heat tolerant than other breeds. Of particular relevance for the present paper, their milk has a higher milk fat and thus energy content, as well as higher protein content and manufacturing quality (CAS, 1978). Therefore, Jersey milk has more value and Jersey cows often produce more milk energy per kilogram of body weight than her larger cousins. This high milk energy output is generally accompanied by a greater DM intake per

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kilogram of BW than for the Holstein and other large frame breeds. Other than a lower yield per cow, there are few 'however's', but Jersey bull calves typically have less value and the breed tends to have more susceptibility to milk fever. In addition, they are reported to have a higher maintenance energy requirement.

What are the nutritional issues for the Jersey? Is she simply a mini-Holstein or should she be given special considerations when formulating rations? Evidence suggests that there are certain nuances to the Jersey that should be considered, but the experimental evidence forming the basis of Jersey 'guidelines' is often sketchy, anecdotal, or based on practical experience, thus harder to document. Issues to be considered in the present paper include:

1. Feed efficiency of Jersey cows compared to other breeds,
2. The potential effect of high intake levels on digestibility,
3. Effective fibre requirements for high milk fat synthesis,
4. Susceptibility to milk fever and transition management,
5. The impact of high milk energy yield on energy metabolism, and
6. Do Jerseys have a higher maintenance energy requirement?

### Feed Efficiency

Whilst Jersey cows produce less milk than Holstein cows, relative to BW or 'metabolic' BW ( $BW^{0.75}$ , or MBW), milk energy output is often higher in the Jersey. These comparisons are obviously affected by the genetic potential of the individuals, as considerable variation in potential yield exists within breeds as well as between breeds. For example, the recent highest 365-day production data available on the internet were 38,570 and 75,275 lb for a Jersey and Holstein, respectively. Assuming a BW of 425 kg (935 lb) for the Jersey and using the 800 kg (1760 lb) BW reported for

the Holstein, the milk yield per kilogram of BW is virtually the same. However, fat content of the Jersey milk was more than double that of the Holstein, while milk protein content was increased by half in the Jersey. Thus, on a solids-corrected or energy basis, the yield per kilogram of BW is higher for the Jersey. Relative to MBW, which relates to surface area and relative heat dissipation, the energy-corrected milk yield of the two breeds is more similar.

In a summary of the U.K. dairy industry (CAS, 1978), the feed efficiency of Jersey cows and Friesian cows were compared based on milk solids output relative to total metabolizable energy intake required for maintenance and production. On this basis, there was a slight advantage for the Jersey. However, when compared on the basis of the maintenance requirement of the Friesian and the current market value of the milk, the net value of Jersey solids output was greater than for the Friesian. In another comparison, Oldenbroek (1988) compared the use of feed net energy for milk energy output in the Danish Jersey and three large frame Dutch breeds fed either all roughage or 50% concentrate diets. In this comparison, which used relatively low yielding cows, the Jersey produced more lactation energy from forage. In addition, the Jersey produced more milk energy relative to maintenance energy requirements, regardless of diet. However, in both cases, the comparisons are based on a common maintenance requirement per kilogram of BW, and there are a number of reports in the literature suggesting the Jersey has a higher maintenance energy requirement than the Holstein (e.g. Solis et al., 1988).

### Intake and Digestibility

In the study by Oldenbroek (1988), the DM intake of the Danish Jersey studied was 3.8 to 3.9 % of BW, whilst that of the relatively low yielding large frame cows was only 3 to 3.2% of BW. Relative to the larger cows, intake of the Jersey was 83%, whilst BW of the Jersey

was roughly 75%. Thus, even in low yielding Jersey cows, intake can not be predicted from BW using equations developed for Holstein cows (Holter et al., 1997). It is not uncommon for intakes equalling 5% of BW to be achieved in Jersey cows. As increased intake depresses the digestibility of many dairy rations, one concern is that diet digestion may be depressed to a greater extent in the Jersey than assumed for the Holstein. However, in a study employing a limited number of observations, rate of passage measured in Jersey cows was higher than for Friesian cows, but diet digestion was not different for the two breeds (Ingvarsten and Wiesbjerg, 1993). Faster passage rate may be a consequence of higher intake levels driven by greater milk energy output. On the other hand, faster passage rate may allow greater intake and be a consequence of more efficient mastication and rumination in the smaller breed (Dürst et al., 1993). Alternatively, faster passage rate may simply reflect a genetic difference related to the rumen size and anatomy of two breeds.

### **Milk Fat Composition and Ration Fiber Content**

The higher fat content of Jersey milk requires more precursors for milk fat synthesis. Comparisons of the milk fatty acid composition of Jersey and Holstein milk (Beaulieu and Palmquist, 1995; Bitman et al., 1996; Drackley et al., 2001) revealed that Jersey milk fat had a higher content of shorter chain fatty acids, but there was little difference in total long chain fatty acid content. However, differences in the response of C18:0 and C18:1 to treatments did suggest that mammary activity of  $\Delta^9$  desaturase was lower in Jersey compared to Holstein cows (Beaulieu and Palmquist, 1995; Drackley et al., 2001). This enzyme converts C18:0 (stearic acid) to *cis*-9 C18:1 (oleic acid) and *trans*-11 C18:1 (vaccenic acid) to *cis*-9, *trans*-11 C18:2 (the predominant conjugated linoleic acid in milk) in the mammary gland. Unlike the long chain fatty acids which are derived from blood, the mammary gland must synthesize the short

chain fatty acids from acetate and  $\beta$ -OH-butyrate. Thus, higher milk fat in Jersey milk is in part accomplished by greater endogenous fat synthesis by the mammary gland, which requires lipogenic volatile fatty acids (VFA) from the rumen. The proportion of the lipogenic VFA absorbed is promoted by forage digestion; therefore, recommendations in the popular press frequently stress the need for 'effective fiber' in Jersey rations (e.g. Macmillan, 1996 citing McCollough). While this logic is sound, supporting data are not evident.

In contrast, other popular press articles (e.g. Etchebarne, 1999; Calvin Covington, personal communication) state the opposite. Based on observations working with California herds, Etchebarne (1999) suggests that Jersey cows can, and should, be fed diets containing less fiber to insure that their maximal DM intake can be achieved. The author asserts that lower fiber diets can be fed to the Jersey without the drop in milk fat content and health problems arising from rumen acidosis that would occur if the same diet was fed to the Holstein, implying a greater tolerance to high levels of dietary starch. Evidence in the scientific literature appears to support the latter premise. Research with low-forage diets designed to cause milk fat depression observed less depression in Jersey than Holstein cows (Jorgensen and Schultz, 1965; Annison et al., 1974), but the studies were not designed to test breed differences. In more recent studies, variations in source and content of neutral detergent fiber (NDF) and nonstructural carbohydrate (NSC) of rations fed to Jersey cattle were not associated with differences in milk fat content or yield (Elliot et al., 1995; Harmison et al., 1997; West et al., 1997; Kauffman and St-Pierre, 2001). There was also no effect on milk fat percentage of Holstein cows when soybean hulls replaced ground corn in the study of Kauffman and St-Pierre (2001), but the low NDF level was not extreme. West et al. (1997) observed a depression in DM with increased inclusion of Bermuda grass hay in rations fed to Jersey cows. This depression in

DM intake was not observed in Holstein cows, supporting the concept that, depending on fiber source, Jersey cows may need lower NDF levels in their ration to achieve maximal DM intake (Etchebarne, 1999). Further confusing the issue, milk fat percentage was decreased when the proportion of alfalfa and Bermuda grass hay was increased in rations fed to Jersey cows (West et al., 1997). Obviously, these issues need clarification.

### Milk Fever and Transition Diets

There are a number of studies which have documented a higher incidence of milk fever in the Jersey (Horst et al., 1997). This has been suggested to be related to a reduced number of  $1,25(\text{OH})_2\text{D}_3$  (the active form of vitamin D) receptors in the intestine which are responsible for increasing calcium absorption in early lactation (via increased calcium binding protein activity). The activation of vitamin D is stimulated by parathyroid hormone, but the effectiveness of parathyroid hormone is inhibited as blood base levels increase. Current thinking is that cationic rations increase the incidence of milk fever by limiting the ability of parathyroid hormone to up-regulate calcium absorption via vitamin D receptors in the gut. For this reason, it has been recommended that target urine pH levels when adjusting the cation-anion difference of transition rations be lower for Jersey (5.8 to 6.2) than for Holstein cows (6.2 to 6.7). The higher acid load may compensate for lower vitamin D receptor numbers in the gut of the Jersey, but acidic diets may have negative effects on metabolism and health, thus these recommendations may also need further clarification.

### Energy Metabolism

Do Jersey cows have a higher maintenance energy requirement? As the efficiency of milk energy synthesis increases with milk fat content, is the efficiency of metabolizable energy (ME) use for lactation

greater for Jersey cows? Do high levels of intake affect digestion or influence gaseous or heat energy loss? Limited calorimetric comparisons of the two breeds from the first half of the century suggested little difference between the Jersey and Holstein in terms of energy metabolism (Ritzman and Benedict, 1938; Brody, 1945), but studies in nonlactating animals showed a much higher maintenance requirement for the Jersey (Solis et al., 1988).

A study comparing the energy metabolism of Holstein and Jersey cows was conducted at the USDA Energy Metabolism Unit in Beltsville, MD in cooperation with the USDA ARS Experiment Station (a Jersey herd) in Lewisburg, TN and supported in part by the American Jersey Cattle Association (Tyrrell et al., 1990 and 1991; Bitman et al., 1996). The study compared the energy metabolism of Jersey and Holstein cows fed total mixed rations without or with supplemental fat from cottonseed (Table 1). Three measurements of energy balance were obtained in eight lactating cows of each breed at 7, 23, and 39 weeks after calving. In addition, measurements were obtained on four dry cows of each breed to determine basal levels of digestion and metabolism. The relative yield potential of the two breeds was similar, as fat-corrected milk yield per kilogram of MBW at peak lactation and DM and energy intakes per kilogram of MBW throughout the study were not different between breeds. Surprisingly, digestibility of energy was higher in the Jersey. This was countered by slight increases in urine and methane energy loss, such that ME intake was not different. The higher milk fat content of the Jersey was associated with numerically higher milk energy (as a percentage of intake energy), while tissue energy retention was numerically lower such that energy balance was not different between breeds. On a MBW basis, the incremental relationship between ME intake and net energy (NE) for lactation (milk energy corrected for tissue energy loss or gain) was similar between the two breeds. However, the

breeds differed slightly in the partitioning of ME between milk and body tissue. A similar conclusion was reached when measurements of the energy metabolism of lactating beef cattle was compared to previous measurements from Holstein cattle (Reynolds and Tyrrell, 2000).

Based on the regression of NE on ME, the data obtained from the lactating cows did not suggest any difference in the maintenance energy requirements of lactating Jersey and Holstein cows. However, the validity of using of the regression of NE on ME to estimate maintenance energy requirements is often questioned. In feeding the dry cows for this study, the maintenance requirement of Holstein cows was used to estimate energy requirements for maintenance of the dry Jersey cows (120 kcal/kg MBW). This level of feeding was quickly increased by 30% to slow dramatic weight loss in the Jersey cows. The energy balances in Table 1 show the higher ME intake required to avoid tissue energy loss in the Jersey cows. Reasons for this difference are not certain, but may in part be due to differences in body composition as the Jersey tended to be leaner, and numerous studies have shown that maintenance requirements decrease with increasing body fat composition.

### Conclusions

1. Dry matter intake per kilogram of BW is often higher in Jersey cows, reflecting greater milk energy yield relative to BW. This may be accompanied by faster passage rate of digesta through the gut, which could be a consequence of greater intake. Alternatively, more effective mastication and rumination in Jersey cows may increase passage rate and allow more intake. Regardless, in a limited number of studies, higher relative intake in Jersey cows does not appear to reduce diet DM digestibility compared to Holstein cows.

2. The effective fiber requirements of Jersey cows have been suggested to be more stringent than that of Holstein cows. Alternatively, the Jersey is suggested to be more tolerant of high dietary starch levels. These considerations may be related to differences in rate of passage between Jersey and Holstein cows and need clarification.
3. Maintenance energy requirements are higher in dry Jersey cows compared to Holstein cows. Reasons for this difference are not apparent, but body composition may play a role.
4. In a calorimetric comparison of the energy metabolism for Jersey and Holstein cows, the Jersey used slightly more dietary energy for milk and less for tissue, but on a MBW basis, the use of ME for NE was remarkably similar for the two breeds.

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**Table 1.** Production and energy metabolism in Jersey and Holstein cattle<sup>1</sup>.

	Jersey		Holstein		Breed effect
	Lactating	Dry	Lactating	Dry	
Average performance					
DM intake (kg/day)	15.9	4.8	22.0	6.4	P < 0.01
Body weight (kg)	402	364	636	738	P < 0.01
Milk yield (kg/day)	21.1	-	32.9	-	P < 0.01
Milk fat (%)	5.10	-	3.86	-	P < 0.01
Milk protein (%)	3.68	-	3.22	-	P < 0.01
Energy intake					
Intake (Mcal/day)	304	72.6	100.2	-	P < 0.01
Intake (Kcal/BW <sup>0.75</sup> )	3.41	814	799	-	NS
Energy metabolism (% of ingested energy)					
Digested	65.2	-	64.1	-	P < 0.10
Metabolizable	56.3	-	55.4	-	NS
Milk	23.6	-	21.6	-	P < 0.10
Tissue	0.8	-	2.0	-	NS
Energy balance	24.3	-	23.6	-	NS
Energy metabolism (Kcal/BW <sup>0.75</sup> )					
Intake	-	261	-	205	-
Digested	-	183	-	138	-
Metabolized	-	151	-	113	-
Tissue	-	-0.6	-	-1.5	-

<sup>1</sup>Tyrrell et al. (1991) and unpublished observations.





## Managing Feed Costs During Low Milk Prices

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### Introduction

Producers all around the world are interested in making a decent return on their investment in dairy production. Milk prices have steadily decreased over time and producers must make the appropriate decisions to maintain or improve profitability. An immediate response to this type of scenario is to control input costs. However, there are several ways (some appropriate and others not) in which “cost” should be controlled. Furthermore, producers and consultants often question the profitability of higher production and suggest that lower production levels are more profitable based on observational analysis. The purpose of this presentation is to review these concepts with specific emphasis on feed cost.

### Controlling Cost

There are several ways in which cost can be controlled. One strategy might involve finding the same inputs at cheaper sources – we call this cost efficiency control. Another might involve selecting a “different level of input” and therefore indirectly selecting a different level of production as a target. In this second strategy, overall feed cost are indeed controlled, but the production level also changes as a consequence of the decision choice. Each of these strategies will be described and discussed.

### Cost Efficiency Control

The basic assumption of the strategy to improve feed cost efficiency is that the production response to inputs is fixed and one is merely finding a cheaper source of nutrients. As input cost per unit is reduced and/or product value per unit increased, profits will increase. Ferguson et al. (1987) and Galligan et al. (1990) reported an approximate 15% feed cost savings on herds subscribing to a routine ration formulation program. These relatively small herds found cheaper sources of grain inputs through the selection of commodity feeds.

Several methods have emerged to help identify “efficient” sources of feed inputs:

a) Petersen method – The basic assumption of this methodology is that the value of a feed ingredient is the sum of the economic values ascribed to its nutrient content. Often energy and protein are used as the major nutrients to determine a feed’s value. Economic estimates of the value of energy and protein are estimated from the value of soybean meal and shelled corn, feeds that are commonly purchased (Petersen, 1932). However a limitation to this methodology is that it does not ascribe value to “nutrient density”.

b) Regression methods are extensions of the Petersen method. With these methods, more than two feeds can be used as references to

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estimate the nutrient values. Secondly, multiple nutrients can be used to establish the value of a feed ingredient (St-Pierre and Glencoe, 2000).

c) Linear programming methods are perhaps the most robust and widely used and are found in ration formulation programs (e.g. Spartan and DairyLP). This method establishes the value of a feed based on a number of dimensions (nutritional, feed availability, feed ratios, etc) and calculates a “reduced cost” for each potential feed ingredient. This reduced cost value is based on the hypothesized biological response (i.e. the biology described as nutrient constraints in the linear program of the ration formulation software). Secondly, non-nutrient constraints, such as the availability of certain feed ingredients or feed ratios, are considered as well. Lastly, the nutrient profile of a feed or “the fit” of a feed into a ration is also considered in the determination of these values. Reduced cost estimates for feed ingredients, indicating the change in feed cost that could occur before a different ration is used, is optimal.

d) Non-linear programming methods (e.g. CPM dairy) do not have “reduced cost” as direct outputs. Users must vary parameters of interest and see the changes in the response function (ration cost) to estimate reduced cost.

Other options to “control feed cost” occur when herds increase in capacity and are able to bulk purchase feed ingredients at reduced rates. Small producers can organize into purchasing groups to also capture some of these economies of scale benefits.

Feed inventory management is yet another method to control feed cost by reducing inventory cost. These costs are scale dependent, with larger herds enjoying greater benefits from these strategies.

In short, cost efficiency should always be sought after regardless of the price of milk.

One has to be careful that cost control is not done to the point where production is compromised.

### **Controlling the level of input to also control production**

There are two approaches to control the level of input: a) lower the level of production for which the ration is balanced for and thereby reduce the input feed cost, and b) introduce additional “groups” of cows to control the degree of over and underfeeding and thereby control overall feed cost.

Behind both of these approaches is the question as to whether the production of additional milk is profitable and what is the milk response of cows to over and under feeding. One has to be careful to use marginal cost of production rather than average cost of production to evaluate this question. Production curve theory tells us that optimal production occurs when the marginal cost of production equals the marginal return of the product produced. Production below and above this level will yield a lower level of profit. What is the marginal cost of production and how does it differ from average cost of production?

In any production system, there are fixed and variable inputs associated with making a product. Fixed costs are those that will not change with a decision choice or an increase in a variable input and thus can be ignored in the decision process. However, fixed costs are included along with variable cost in average cost calculations. It is this inclusion of “fixed cost” that makes average cost of production problematic in tactical decision making (i.e., should I feed for a higher production or not). In the context of nutrition and at the cow level, the maintenance cost of the animal is essentially “fixed” – it is the same whether she is producing 1 kg of milk or 50 kg. Under U.S. conditions of production, this cost is approximately \$1.25 to \$1.50 (slightly lower than feed cost per day of a dry cow). In Figure 1, there are two levels of

production and the corresponding ration cost, which would include the maintenance feed cost plus the feed cost associated with production (levels are figurative to facilitate the understanding of the concepts). Ration cost divided by the level of milk yield gives an average feed cost per pound of milk produced, then multiplied by 100 provides the commonly used feed cost per cwt of milk. The profit (income over feed cost) is also calculated. While the average cost per cwt for the 90 lb/day of milk production is considerably higher than the 80 lb/day of production, the higher production level yields a greater profit (\$7.25 vs. \$7.00). Controlling average feed cost per cwt would not lead to profitable production.

What is important is the cost of “marginal” production. The “marginal” cost of producing at 90 lb/day versus 80 lb/day is \$4.00-\$3.00 or \$1.00 per 10 lb of milk. The marginal revenue of 90 lb versus 80 lb per day of milk is the value of 10 lb of milk or  $\$12.50/\text{cwt} \times 10 = \$1.25$ . The marginal profit is therefore \$0.25 for the additional 10 lb of milk. As long as the marginal cost is less than the marginal revenue, higher profits will be realized by increased production.

What are typical marginal cost estimates? One hundred rations (amount and type of feeds) and corresponding milk levels were recorded from nutritional studies published in the *Journal of Dairy Science*. For moderate levels of milk production 60 to 80 lb/day, feed cost was estimated at about \$0.03/lb of milk (Figure 2). As production increases above 80 lb/day of milk, the marginal cost of production may increase due to: a) the need to use more costly feed inputs (feeds with higher nutrient densities), b) increase in passage rate relative to digestion, and c) an increased use of body weight as a nutrient source. However, at moderate levels of herd production, the feed cost of \$0.03/lb of milk can be viewed as relatively constant.

Body weight as a nutrient can have a dramatic effect on the marginal cost of production. One could argue that at the higher levels of production (weight loss is occurring to some degree), the true ration cost (current + the cost of weight loss) are actually higher than reported. Conversely, at the lower levels of production, the ration costs are lower in that some of the nutrients are used for weight gain. These issues taken together would make the estimate of the marginal cost of production (\$.034/lb) a low figure.

Another approach to looking at the marginal cost of production is to investigate a series of rations formulated for different levels of production and then calculating the marginal changes in cost. In Table 1, rations were formulated using CPM Dairy as a ration formulation program. Three sets of rations based on different forages (50% corn silage/50% haylage, 100% haylage, and 100% corn silage) were formulated for increasing levels of production. Marginal cost per pound of milk were calculated as outlined above.

In general the 50/50 forage based ration had the cheapest marginal cost of production. As production increases, the marginal cost of production increases from \$0.03/lb to higher levels, reflecting the use of more expensive ingredients, as well as the effects of an increasing passage rate on the digestibility of feeds. The decrease in marginal cost at the highest level of production reflects inconsistent attributes of production functions and which reflects changes in nutritional programming constraints (i.e., relaxing constraints) to obtained solutions (perhaps these changes are “arbitrary”).

From a feeding perspective, it appears that higher production levels will yield higher profits. Higher production is often associated with “disease concerns or reproductive inefficiency”, and thus, producers claim that in addition to feed cost, other costs increase, thus inflating the marginal cost of production. A

review of the epidemiological literature (Fetrow, 2002) does not see this causal relationship of higher production and disease, and the effects of high production on reproduction appear to be small and controllable (Grohn and Rajala-Schultz, 2000). These associations of production and disease/reproductive inefficiency are perceived associations and are due to the greater concern bestowed on high producing cows compared to low production cows (i.e., a sick high producing cow is more likely to be seen by a vet than a low producing cow). More attempts at rebreeding, higher producing cows than lower producing animals, as well as more health care dollars spent on diseased animals, fosters this perceived casual association.

### Summary

To be profitable in dairy production, producers must understand the economic basis of changes in their production system. All herds, at all times, should practice cost control efficiency as long as production is not impaired. For the economic evaluation of a given decision, they must know the marginal changes in cost and revenue to make sound choices. In general, a marginal cost of higher production at \$0.03 to \$0.06/lb of milk can be used as an industry estimate. At higher levels of production, this marginal cost should theoretically increase. The point where the marginal cost of milk production equals the marginal return of production will define the limit of production that maximizes profit (income over feed cost).

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**Table 1.** Feed costs and marginal cost of production as affected by milk production and type of forage.<sup>1</sup>

Milk, lb/day	Feed Cost/Cow/day			Marginal Cost of Production (\$/lb milk)		
	CS/Haylage	Haylage	Corn Silage	CS/Haylage	Haylage	Corn Silage
40	\$2.51	\$2.46	\$2.07	\$0.00		
50	\$2.77	\$2.76	\$2.39	\$0.03	\$0.03	\$0.03
60	\$3.06	\$3.32	\$2.76	\$0.03	\$0.06	\$0.04
70	\$3.52	\$3.74	\$3.24	\$0.05	\$0.04	\$0.05
80	\$3.92	\$4.22	\$3.75	\$0.04	\$0.05	\$0.05
90	\$4.31	\$4.81	\$4.17	\$0.04	\$0.06	\$0.04
100	\$4.62	\$5.02	\$4.66	\$0.03	\$0.02	\$0.05
110	\$4.95	\$5.29	\$5.15	\$0.03	\$0.03	\$0.05

<sup>1</sup>Rations were formulated using CPM Dairy. Rations consisted of 50% cornsilage (CS) and 50% haylage, 100% haylage, or 100% corn silage, respectively.

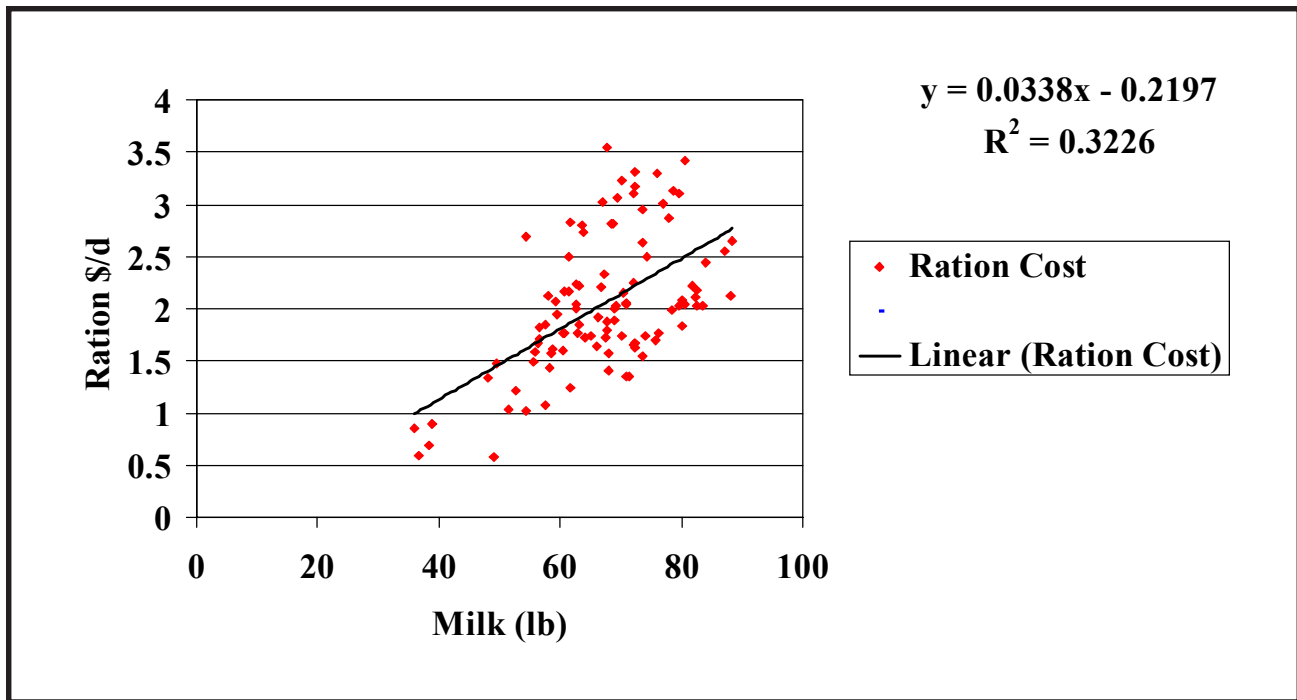
Milk Yield (lb/day)	Feed Cost (\$/day)	Feed Cost/cwt	Profit (\$/day)
80	\$3.00	\$3.75	\$7.00
90	\$4.00	\$4.44	\$7.25

**Milk is valued at \$12.50/cwt.**

**Profit = income over feed cost.**

**What level of production is best: 90 lb.**

**Figure 1.** Calculations to demonstrate that average feed cost per cwt (or lb or per kg) of milk can be misleading.



**Figure 2.** Relationship between 4% fat-corrected milk yield and ration cost (100 rations used).

## Managing N, P, and K for the Dairy Industry

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### Abstract

Challenges in meeting environmental objectives continue to dictate that new and innovative strategies be developed, such that economically viable practices are implemented. Nutritional regimens drive the input side of nutrient management. Therefore, adoption of feeding practices that minimize excretion are necessary. Minimizing over-feeding of phosphorus has been demonstrated to reduce P excretion. Similar findings have been observed when protein feeding is tailored to not exceed animal needs. Current regulations enhance the challenge of utilizing co-product feeds while minimizing nutrient excretions. Yet, regulations currently in place in some states, with the opportunity to be adopted in others, require that we address the challenges now and that we not expect a post-excretion solution.

### Introduction

Environmental issues are not going away any time soon. Greater emphasis on animal production impacts on the environment has resulted in greater scrutiny of practices and methods to mediate impacts. Historical approaches have addressed post-excretion strategies, while more recently, the role of nutrition has received an increasing degree of attention. As we continue to minimize the pollution potential of animal agriculture, the nutritionist will have greater involvement in an

effort to develop pre-excretion solutions. We've seen the signs for this in the EPA-USDA Animal Feeding Operations (**AFO**) Strategy, whereby the concept of a Comprehensive Nutrient Management Plan was introduced. These plans incorporated the option of including feed management as a component of a farm's environmental plan. To take this a step further, the Natural Resources Conservation Service (**NRCS**) is currently developing a Conservation Practice Standard for feed management. Once adopted, the standard becomes part of NRCS's technical manual and will further elevate the attention nutrition strategies receive, incorporating the nutritionist as a standard member of the nutrient management team.

### Regulatory update on nitrogen and phosphorus

On December 16, 2002, much of the livestock sector breathed a sigh of relief. The new Concentrated Animal Feeding Operation's (**CAFO**) regulations, a revision of the Clean Water Act, had been signed and appeared to be quite fair, leaving much of the details up to individual states to work through. The final rule provided state flexibility by:

- Allowing states to tailor their permit program to address specific state needs, taking into account the size, location, and environmental risk posed by individual operations,

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- Authorizing states to determine that specific CAFO have no potential to discharge under any circumstances, thereby not needing permits,
- Providing flexibility for states to tailor nutrient managements, and
- Authorizing states to adopt alternative performance standards to promote innovative technologies.

One of the greatest concerns over the two years during which time the revision process ensued was how land application of animal manure would be handled. The final rule requires “CAFO to develop and submit a certified Permit Nutrient Plan, which would be reviewed annually, recertified every five years, and would have limited manure spreading on all land owned or under the operational control of the CAFO to the nitrogen-based rate, unless soil or other field conditions at the CAFO warranted limiting the application rate to the more stringent phosphorus-based rate” (Federal Register, Vol. 68, No. 29, February 12, 2003). However, Michigan and Ohio have been using P-based planning practices for a number of years, and Indiana considers P-based nutrient planning on a case-by-case basis.

A couple of changes to the previous CAFO rule do affect the dairy industry. One is the inclusion of stand-alone heifer operations in the definition of a CAFO. Those operations with greater than 1000 head are designated as CAFO and must apply for a permit. The new rule also eliminates the 25-year, 24-hour storm event exemption that previously allowed operations that only discharged during such storm events from applying for a National Pollutant Discharge Elimination System (**NPDES**) permit. This change means that all operations of CAFO size must apply for a permit regardless of whether or not they only discharge under 25-year, 24-hour storm conditions. As a result, a greater number of operations will be permitted. However, the containment standard for a 25-year, 24-hour storm event is retained. Finally,

the new rule clarifies that runoff from land as a result of manure application from a CAFO is a discharge and therefore subject to NPDES permit requirements.

Many state Natural Resource Conservation Services (**NRCS**) have developed a Conservation Practice Standard 590, often referred to as the P Index. The P Index is a risk assessment tool that can be implemented as a mechanism for determining manure application rates. However, the P Index, itself, does not designate application rates. Rather, it is up to the state to take the calculated risk factors and develop a scaling system whereby operations that fall within a range of risks are allowed to apply manure at a state-determined application rate. While the EPA’s new CAFO regulations do not require P-based manure application or use of the P Index, it is likely that states with a P Index developed will continue to proceed with implementation of the index and other states will continue to develop one. Therefore, as nutritionists, we need to continue to be aware of the challenges in maintaining a nutrient balance and of the implications that nutritional strategies have on environmental risk.

### **Nutritional management of phosphorus**

Recently, a number of studies have reported that overfeeding dietary P, by as much as 50%, is a common practice (Beede and Davidson, 1999; Wu *et al.*, 2001). Beede and Davidson (1999) discussed several reasons for overfeeding, including addition of safety margins beyond the recommended dietary P in order to adjust for inaccuracies with recommendations or uncertainty with the proportion of dietary P that is available. Improved milk yield and reproductive performance were discussed as reasons for overfeeding as well. Beede and Davidson (1999) go on to establish that each of these reasons is a misconception. Trials have demonstrated that such practices result in quadratic increases in P excretion (Morse *et al.*, 1992). Morse *et al.*

(1992) observed an increase in fecal P excretion of 48.6% when lactating cows were fed diets containing 36.5% greater P. Knowlton and Herbein (2001) observed linear increases in fecal and urine P excretion with increasing dietary P from 0.34% to 0.51% to 0.67% dietary P. Apparent digestibility of P in early lactation Holsteins decreased quadratically with increasing dietary P (Knowlton and Herbein, 2001). Wu *et al.* (2000) found a 23% reduction in fecal P excretion by reducing the dietary P content from 0.49 to 0.40% for mature lactating Holstein cows.

The interest in feeding co-products to dairy cattle and other livestock has surged in the past couple of years as a result of increased interest in ethanol production in response to fossil fuel costs. Particularly in the Midwest, ethanol co-products have become an economical, available feed source. However, corn co-products are higher in phosphorus than corn and soybean meal (Table 1). While, in many cases, these feeds are less expensive than corn and soybeans, thereby reducing diet cost, cost of manure application to more acres should be considered when determining the economics of feed sources. Co-products serve as an important feed source for the animal industry. Producers and nutritionists need to be aware of product nutrient content and the availability of nutrients in order to appropriately manage excreted nutrients. Variability in feed source, both between processors and within a processor, necessitates that each batch of feed be analyzed and ration formulations adjusted accordingly in order to minimize overfeeding of nutrients. Using a feedlot example, it becomes obvious that appropriately managing manure nutrients requires some forward thinking. Table 2 illustrates calculated land needs to maintain a phosphorus balance between P excreted and P harvested by corn silage. The calculations are based on research data collected by Wu *et al.* (2000) and that estimated from a simulation that incorporates corn co-products in the diet and estimates excretions based on the relationship

between intake and excretion observed by Wu *et al.* (2000). The simulation shows that incorporation of corn co-products does increase the land needed to maintain soil P. However, P excretion of the example diet that includes the corn co-product remains below that observed by Wu *et al.* (2000) when mineral P was included in the diet beyond that recommended by NRC (2001). In the examples provided, it is assumed that manure is applied only on corn silage. Therefore, more silage is grown than that consumed by the animal (20% of dietary DMI) in a year's time. In reality, producers feeding both corn silage and alfalfa silage may grow the alfalfa silage as well, in which case, the corn silage grown would match feed inventory needs and alfalfa fields would utilize the remaining manure. One factor that can't be overstated is that as we move towards P-based nutrient management planning, the benefit of having manure handling systems designed to retain N rather than lose N to the atmosphere through volatilization becomes obvious. In all three examples provided in Table 2, supplemental N is needed to grow the indicated quantity of corn silage. So, while in the past, manure handling systems sought to lose N in order to reduce the land needed to spread manure nutrients using N-based nutrient planning, volatilization becomes a costly process when managing nutrients under P-based planning.

### What about K?

Potassium is not an environmental concern from a human health perspective. Rather, soils rich in K can contribute to high forage K concentrations as a result of luxury consumption. The result can be grass tetany for the animal that consumes the forage. The dairy cow's minimum requirement for K is 0.90 to 1.0% of the ration DM (NRC, 2001). The maximum tolerable level is about 3.0%. Because more K is lost through sweat and saliva, supplemental K can help to alleviate symptoms of heat stress. Research results have been variable, but increasing dietary K levels to 1.5

or 1.6% of ration DM during periods of heat stress may be beneficial to the cow. Excessive K intake can lead to udder edema in fresh cows, greater incidence of retained placenta, and greater risk of displaced abomasum. Excessive K intake decreases Mg absorption, decreases feed intake and milk production, increases water intake, and increases urine output. High levels of dietary K during the dry period can predispose the fresh cow to milk fever, DA, uterine problems, and other metabolic disorders. Potassium supplementation is seldom needed in forage-based diets because most forages contain high concentrations of K. As with most nutrients, feed sampling and analysis is recommended to avoid over-supplementing diets with mineral sources of K. In the case of K, forages take up far more than needed for maximum DM yields per acre. Plant levels of 2.0 to 3.0% are adequate for plant growth, but 6.0% K in grass silage has been reported. Excessive feeding of K puts substantially more K into the environment than necessary.

### What's ahead?

In the past decade, most of the emphasis placed on environmental decisions has been based on water quality concerns. However, nuisance issues, primarily odor, are often the trigger that stimulates controversy regarding animal production. It is no surprise, then, that we are seeing greater emphasis placed on air emissions from animal agriculture. Some states have implemented odor regulations, while other states now have standards for specific gases or classes of gaseous compounds. For example, in 1997, the California South Coast Air Quality Management District (SCAQMD) established emission reduction goals for animal agriculture within their area in response to non-attainment of  $PM_{10}$  (particulate matter) and ozone standards (CM#99WST-01 Appendix B: New and Revised Stationary Source Control Measures). The SCAQMD has set a goal of 30% reduction of volatile organic carbon emissions from livestock waste by 2006 and 50% reduction of ammonia

emissions from dairy operations by 2006. One of the primary mechanisms for reaching the stated ammonia goal is relocation of the dairy industry out of the area. However, if the targets are not met by January 1, 2004, dairy and other livestock facilities still located in the Basin will be subject to ammonia controls. Possible controls that were indicated by SCAQMD include reduced nitrogen feeding, promotion of aerobic manure storage conditions including use of enzymes and microbial products, off-site composting, and chemical alteration of manure pH. With the pending revision of the Clean Air Act, it is foreseeable that restrictions may increase at the Federal level as well.

Particulate matter poses perhaps the greatest challenge for animal agriculture. Direct emission sources of  $PM_{10}$ , the coarse particulates, arise from primarily combustion processes (Figure 1; EPA, 1998). Direct emissions of  $PM_{2.5}$ , respirable particulates, are also primarily the result of combustion processes (Figure 2; EPA, 1998). In addition to direct emissions, secondary processes whereby  $SO_x$ ,  $NO_x$ , and  $NH_3$  react in the atmosphere to form ammonium sulfate and ammonium nitrate fine particles, contribute to as much as half of the  $PM_{2.5}$  measured in the U.S. The EPA estimates that 86% of the national ammonia emissions are from miscellaneous sources that include livestock and fertilizer (Figure 3; EPA, 1998). Livestock agriculture accounted for 83% of all emissions in the miscellaneous category with fertilizer application comprising the remainder.

The EPA already regulates many combustion processes through industrial and utility permitting processes. Area sources are not likely to be targeted for future regulation because they are non-point source emitters. To address particulate emission reductions, animal agriculture is a likely target due to its contribution to ammonia emissions which, in turn, can contribute up to half of particulate concentrations and emissions. Animal agriculture must be taking steps to address

ammonia production and emission. In 1984, legislation was enacted in the Netherlands to reduce 2000 ammonia emissions by 50% relative to 1980 emissions (Lenis and Jongbloed, 1999). Such requirements here in the U.S. could dramatically change animal agriculture, both structure and management.

Nutritional strategies currently available to reduce ammonia emissions include lowering dietary protein levels, changing the ratio between urinary nitrogen and fecal nitrogen, reducing urine pH, inhibiting urea degradation, and binding ammonia. Many of these examples have been tested in swine and poultry research; however, the principles should apply to the dairy animal as well. As the issue of reducing ammonia emissions travels across the Atlantic and the U.S., additional innovative strategies will require development and testing. Furthermore, we could see the cost to stay in business change as well.

#### *Reduced dietary protein*

Minimization of nitrogen excretion is the most obvious method to curb ammonia emissions. By reducing the available substrate, less ammonia will be formed and volatilized. Endogenous nitrogen losses are unavoidable. However, steps should be taken to minimize overfeeding of protein and amino acids beyond animal needs. In Holstein heifers, James *et al.* (1999) observed a 28% reduction in ammonia emissions when N intake was reduced 14%. Total N excretion was reduced only 20%. Tomlinson *et al.* (1996) reported an approximate 20% reduction in N excretion associated with a 3% reduction in dietary CP for lactating dairy cows. A greater number of examples of this strategy are found in swine and poultry research. Reduced crude protein (**CP**) in swine grower and finisher diets, supplemented with lysine, methionine, tryptophan, and threonine, resulted in reduced nitrogen content of the manure and reduced ammonia emission rates from the manure (Hobbs *et al.*, 1998). The reduced CP diets contained 16.1% CP in the grower phase

and 14.0% CP in the finisher phase compared to 20.8% and 18.9% CP in the control diets, respectively. Ammonia emission rates were reduced 74% and 65% in the grower and finisher phases, respectively, as a result of feeding the reduced CP diets. In a review, Lenis and Jongbloed (1999) cited work that observed a 15% reduction in N excretion and 17% reduction in ammonia emission following implementation of a 2-phase feeding plan. Greater reductions are possible. Reducing CP content of broiler diets resulted in decreased litter N content but no significant differences in ammonia concentration in the house (Ferguson *et al.*, 1998). However, analyzed diets showed that CP content had been decreased by less than 2 percentage units. The 13.3% decrease in N intake did correspond to 18.2% reduction in litter N content. Elwinger and Svensson (1996) fed broilers diets containing 18, 20 or 22% CP and measured ammonia emissions from the litter bed. Total N losses in the houses averaged 18 to 20% of total N input. A linear trend of increasing ammonia emission with increasing dietary CP content was observed. Increasing dietary CP significantly increased calculated N losses as well. Generally, as a guide, for each 1% reduction in dietary CP estimated, ammonia losses are reduced by 10% in swine and poultry (Sutton *et al.*, 1997; Kay and Lee, 1997; Blair *et al.*, 1995; Jacob *et al.*, 1994; Aarnink *et al.*, 1993). Similarly, Kerr (1995) concluded that, in swine, N excretion is reduced by approximately 8.4% for each one percentage unit reduction in dietary CP. James *et al.* (1999) and Tomlinson *et al.* (1996) showed similar findings in the dairy animal. However, current work demonstrates that as animals are fed closer to true N requirements, further reductions in dietary CP may result in less pronounced reduction in N excretion and ammonia losses. Thus, this issue is really, how close to requirements can we economically achieve? Regardless, for the ruminant, we continue to be challenged with trying to more precisely define the N and amino acid requirements of the rumen and lower gastrointestinal tract, separately, in order to better

feed the animal as a whole. Restrictions on ammonia emissions add urgency to these needs.

#### *Fecal and urinary nitrogen ratios*

Inclusion of fermentable carbohydrates into swine diets has been shown to shift N excretion from urine to feces (Canh *et al.*, 1997b). Fecal nitrogen is less easily degraded to ammonia. Hindgut fermentation can increase fecal nitrogen while decreasing urinary nitrogen. Inclusion of sugar beet pulp into grow-finishing diets at 5, 10, and 15% resulted in a reduction of ammonia emission by 14% for each 5% increase in sugar beet pulp inclusion (Canh *et al.*, 1998). Slurry pH decreased by 0.4 to 0.5 units with each incremental increase in dietary sugar beet pulp content. Tomlinson *et al.* (1996) illustrated that cows fed calcium soaps of long chain fatty acids (Ca-LCFA) excreted greater urine N (g/day) than those fed control diets ( $P = 0.0006$ ). The authors attribute this to reduced microbial protein synthesis in the Ca-LCFA diets with concomitant increase in ammonia conversion to urea and excretion in the urine. Fecal N (g/day) was numerically less in cows fed the Ca-LCFA diets, demonstrating that a shift in N excretion patterns may have occurred. While this study was not designed to examine strategies to shift N excretion in the dairy animal, future studies are needed to investigate dietary manipulation of N excretion to promote reduced ammonia emissions. No literature was found to confirm or refute the theory that as we push ruminants towards higher grain diets, we are increasing ammonia emissions. However, the need to test this theory is evident. The economics of shifting the high producing dairy cow to a diet with a greater proportion of forage needs consideration as part of a whole farm scenario analysis.

#### *pH manipulation of manure by diet*

Sutton *et al.* (1997) observed a reduction in swine manure pH when 5% cellulose was added to the diet. Canh *et al.* (1997a) observed reduced ammonia emissions of 26 to 53% by

including Ca-salts up to dietary Ca levels of 7 to 10 g/kg. Resulting reductions in urinary pH ranged from 1.6 to 1.8 units. Hendricks *et al.* (1997) observed a 37% reduction in ammonia emissions following feeding calcium benzoate to grow-finishing pigs. Kim *et al.* (2000) observed a 30% reduction in ammonia emissions associated with growing pig diets containing a combination of phosphoric acid and calcium sulfate and lesser reduction in emission (17%) when diets contained a combination of monocalcium phosphate, calcium sulfate, and calcium chloride. Relative to the control diets, no ammonia emission reductions were observed when diets contained a combination of monocalcium phosphate and calcium sulfate, despite significantly reduced urinary pH in these animals. This may relate to the buffering capacity in animals fed these diets. Although no work was found that tested pH manipulation of the ruminant animal, investigation of such an approach is worthy of consideration. However, the buffering capacity of the rumen may preclude favorable results.

#### *Ammonia binding, urease inhibition*

Several feed additives claim to reduce nitrogen excretion and ammonia emission potential by binding ammonia or inhibiting urease. Yucca plant extracts have been evaluated by Powers *et al.* (1999) finding that ammonia concentrations in stored dairy manure were reduced. The product fed claimed that urease inhibition was the mode of action. Amon *et al.* (1995) fed a De-Odorase<sup>®</sup>, a yucca extract, to fattening pigs and observed reduced ammonia concentrations in the feeding rooms over a 7-wk period. Ammonia concentration was reduced, on average, 26% in rooms where the extract was fed. Similarly, ammonia emission was reduced 26% in the study. Alltech, Inc. (Nicholasville, KY), the manufacturer of De-Odorase<sup>®</sup>, believes that the mode of action is through binding of ammonia rather than urease inhibition. Dietary inclusion of clinoptilolite and other clay minerals to reduce ammonia emissions has resulted in

variable findings. Kithome *et al.* (1999) observed that application of a layer of 38% zeolite placed on the surface of the composting poultry manure reduced  $\text{NH}_3$  losses by 44%. Amon *et al.* (1997) observed greater ammonia concentration and emission when clinoptilolite was used in broiler houses. However, use of zeolites, as post-excretion amendments or dietary constituents, have long been studied for their effectiveness in binding ammonia. Continued investigation into binding and inhibition strategies is warranted as is delineation of the mechanism of effective strategies.

### Summary

Odor remains the trigger that spurs neighbors into filing complaints against animal production facilities. However, where previously neighbors viewed odor as a nuisance, there is increasing concern by residents that the odor causes negative health effects. So, the motivation to restrict emissions from animal operations has gained momentum. This is combined, then, with EPA's own efforts to meet the National Ambient Air Quality Standards that were established under the 1997 Clean Air Act Amendments, as well as EPA's interest in adding  $\text{PM}_{2.5}$  (particulate matter with an aerodynamic diameter of 2.5 microns or greater) to the list of NAAQS pollutants. Because ammonia serves as the nucleus for  $\text{PM}_{2.5}$  formation, and because the findings of EPA's monitoring studies suggest that animal agriculture is the large contributor to ammonia emissions, we need to continue to target N feeding practices from the standpoint of air quality-related issues, while continuing to address P due to water quality-related issues. Non-traditional approaches will be necessary. Beyond nutrition, we need to be thinking about things such as manipulating the gene that expresses urease production, the role of beta-agonists in environmental plans, and other mechanisms to improve nutrient utilization, including feed processing. As the challenges become greater, so must the breadth of expertise involved in developing solutions.

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**Table 1.** Phosphorus content of typical feedlot feed ingredients.

Feed	P content (% of DM)
Soybean meal	0.70
Corn	0.35
Corn gluten feed	0.90
Distillers grains with solubles	0.70
Condensed corn distillers solubles	1.00

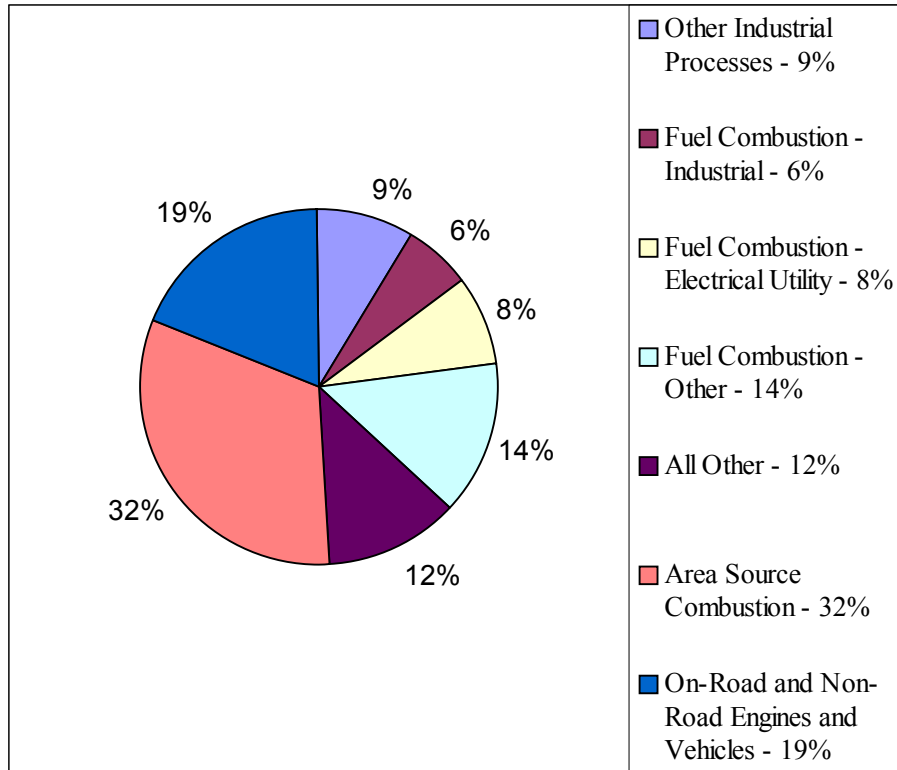
**Table 2.** Phosphorus balance for lactating cow diets with and without corn co-products.

	Wu et al. (2000), 0.31% P	Wu et al. (2000), 0.49% P	Co-product diet
Ingredient, %			
Alfalfa silage	30.00	30.00	30.00
Corn silage	20.00	20.00	20.00
High moisture ear corn	25.50	25.50	12.00
Soybeans, roasted	10.00	10.00	3.00
Distillers dried grains w/ solubles	0.00	0.00	23.50
Bloodmeal	2.00	2.00	2.00
Molasses	5.00	5.00	5.00
Beet pulp	7.00	6.16	4.00
Salt	0.40	0.40	0.40
Mineral and vitamin mix	0.10	0.10	0.10
Monosodium phosphate	0.00	0.84	0.00
Diet composition			
CP, %	17.8	17.8	17.8
Ca, %	0.65	0.65	0.65
P, %	0.31	0.49	0.39
NE <sub>L</sub> , Mcal/kg	1.65	1.65	1.65
Nutrient management factors			
DMI, kg/day	23.0	23.4	23.0
P intake, g/day	72.8	111.8	89.7
Fecal P excretion, g/day	39.9	68.9	54.0
Fecal P, kg/year	14.6	25.1	19.1
Corn hectares (P-basis) <sup>1,2</sup>	0.56	0.96	0.73
Silage consumed, ha/yr (30% DM)	0.17	0.17	0.17
Silage for export, ha <sup>3</sup>	0.39	0.79	0.56

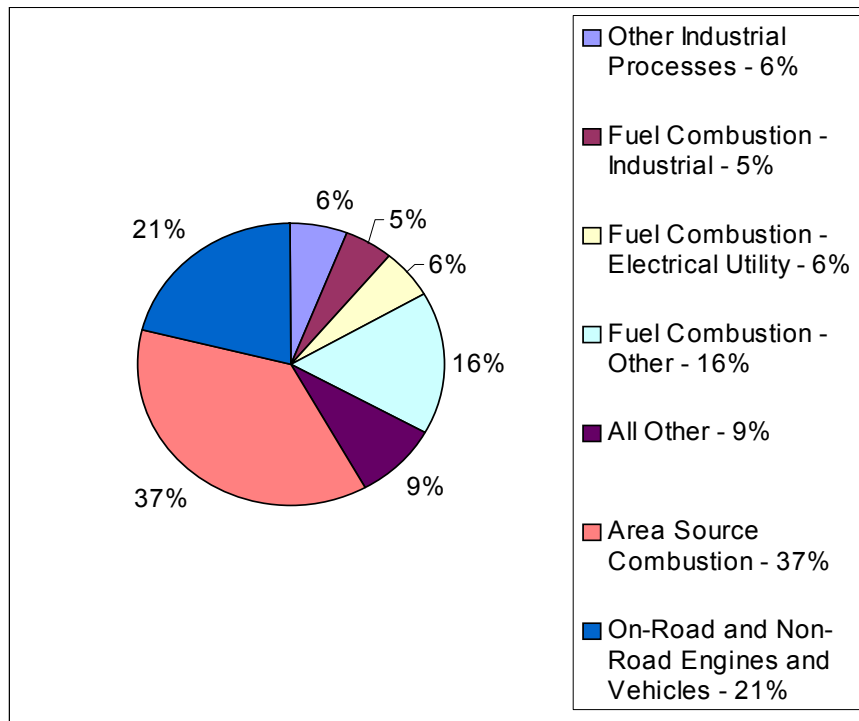
<sup>1</sup>1.75 g P<sub>2</sub>O<sub>5</sub> (0.7777 g P) needed to grow 1 kg of corn silage. Assumed silage yield of 33,695 kg/ha (14.8 T/acre). Therefore, 26.2 kg P needed per hectare of corn silage grown (1 hectare = 2.5 acres).

<sup>2</sup>Assuming harvested at 30% DM, DM yield equals 10,109 kg/ha (23.1 lb/acre).

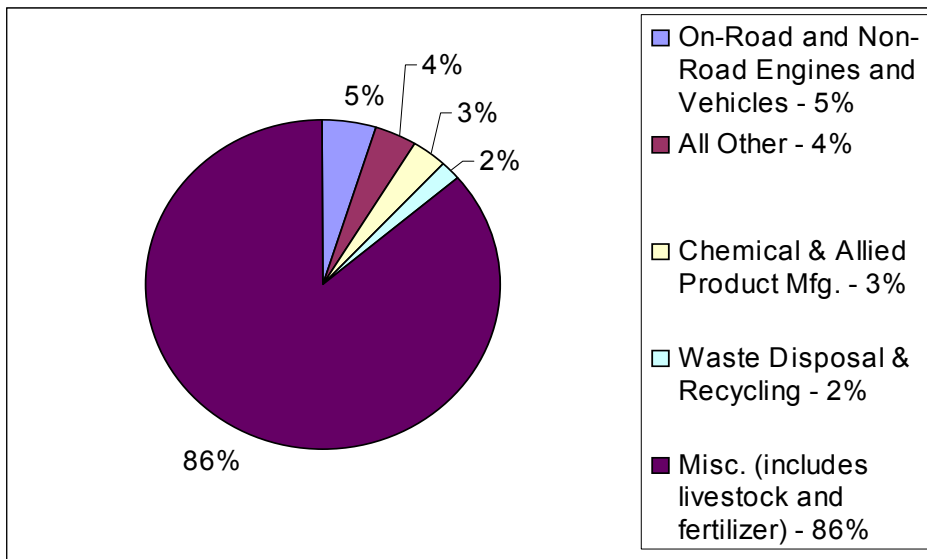
<sup>3</sup>Silage available for feeding to other animals (in addition to the lactating cows) or for sale.



**Figure 1.** Directly emitted national particulate matter (PM<sub>10</sub>) emissions by principal source categories for nonfugitive dust sources (EPA, 1998).



**Figure 2.** Directly emitted national particulate matter (PM<sub>2.5</sub>) emissions by principal source categories for nonfugitive dust sources (EPA, 1998).



**Figure 3.** National ammonia emissions by principal source categories (EPA, 1998).

## Practicalities of Balancing Diets for Amino Acids

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### Abstract

Formulating diets to meet the amino acid requirements of cows is not widely practiced, even though the concept is not new. Where the procedure is accepted because of reasonably accurate formulation tools, the benefits include consistent production performance along with the potential to reduce nitrogen wastage. Amino acid nutrition for ruminants is somewhat more complicated than for monogastrics due to the involvement of rumen microbes. However, microbes provide the greatest potential for meeting requirements and reducing nitrogen losses. This paper provides a practical approach to understanding amino acid nutrition of dairy cows.

### Why Balance Diets for Amino Acids?

There is an urgent need to optimize protein and amino acid use in dairy cows. Excess nitrogen (N), produced by poorly formulating or over formulating the protein portion of the diet, is a burden to both the environment and to the cow. Many producers are following strict guidelines that limit manure application. At the same time, there is pressure to reduce the use of animal byproduct feeds, often resulting in the need to increase ration protein. On the other side of the equation, cows utilize additional energy when converting excess N and amino acids to urea for elimination. Diets need to be formulated to reduce oxidation of amino acids to the extent

possible by feeding the correct amounts of amino acids whenever possible and whenever economical.

Another consideration for using available amino acid technology revolves around animal health. Amino acids are key components of proteins required for the production of enzymes, immunoglobins, some hormones, muscle, and milk. Amino acids contribute to the formation of glucose, which can be in short supply, particularly in early lactation. When the feed fails to supply sufficient amino acids, net catabolism of tissues occurs in order to supply amino acids for the most critical functions. Insuring that the correct amounts of amino acids are available contributes to productive performance by supporting physical and physiological conditions.

Although the benefits of using amino acids is well understood, many feed formulators are lost in the apparent controversy over products and programs available. The purpose of this paper is to remove some of the mystic surrounding this technology and to clear the path for greater application.

### Sources of Amino Acids

In order to meet the cow's need for amino acids, there must be an understanding of not just the needs, but also the supply and the availability of amino acids. The principle sources of amino

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acids are: microbial protein, escape feed protein, and protected amino acids.

### *Microbial protein*

Microbial protein is by far the most important source of protein available to the cow. The amino acid composition of microbial protein has a profile that more closely approximates that of milk and somatic tissue than many of the vegetable protein sources commonly used (Table 1). It is advisable to maximize the production of microbial protein, as it is generally the cheapest source of protein available.

The rumen provides roughly 100 to 150 g of microbial protein/kg of DM consumed (Verbic, 2002). The higher levels are attainable when sufficient nutrients are supplied to support the growth of the microbes. Much like cows, rumen microbes are sensitive to the nutrient supply available to them. Microbial production is influenced by the availability of fermentable carbohydrate (Ahvenjarvi et al., 2002), nitrogen (Verbic, 2002), and minerals (Broudiscou et al., 1999; Carneiro et al., 2000).

Most systems for predicting microbial protein utilize some measurement of energy to predict the amount of protein that can be produced in the rumen (INRA, 1988; AFRC, 1992; NRC, 2001). However, microbial protein cannot be readily predicted from the amount of energy or fermentable carbohydrate supplied (Orskov, 1994; Dijkstra et al., 1998), except at sub-optimal input levels. Growth will be proportionately increased when supply to the rumen is below optimum; however, an over supply does not result in extra growth any more than it does in the cow. In the cow, over supplying feed simply results in greater bunk losses and increased cost. This may explain why microbial efficiency (microbes produced/carbohydrate assumed to be fermented) is often reduced with high grain diets.

Microbial growth and microbial efficiency is improved at higher turnover rates. Turnover rate has long been known to be dependent upon feed intake (Evans, 1981) and high growth rate results in the dilution of microbial maintenance and improved efficiency (Meng et al., 1999). Therefore, insuring that intake is maximized is an ideal system for reducing the need for escape protein from ingredients and gaining the most from the rumen.

The nutritional requirements of rumen microbes may differ from that of the host, particularly in the area of intermediary metabolism. Microbial production may be enhanced or reduced with the addition of minerals, such as sulfur (Carneiro et al., 2000) or phosphorus (Broudiscou et al., 1999). The addition of unprotected fat, particularly if the fat is unsaturated, can inhibit protozoa growth and fiber digestion (Oldick and Firkins, 2000).

Under certain conditions, microbial growth has been shown to be greater than anticipated. This may largely reflect the lack of understanding of the needs of rumen microbes. Miller-Webster et al. (2002) showed that yeast culture improves the digestion capacity of mixed rumen microbes. Improvements in ammonia uptake at some times after feeding, likely associated with increased microbial production, were demonstrated by Ehjalberta et al. (1999). Likewise, other fermentation products have been shown to result in enhanced microbial performance (Hoover et al., 2001).

To insure that microbes get enough soluble nitrogen to support growth, they need at least as much as what is incorporated into microbial protein, plus some extra to account for material that escapes from the rumen before it is converted to microbial protein. The amount lost will increase with rumen turnover rate.

If there is not enough nitrogen in the rumen to support microbes, then feed will not be digested properly. Often the manure will

appear stiff and fibrous. Bear in mind that there also are other causes for this to occur. Results of experiments where the flow of nitrogenous components have been measured suggest that there is a general overage of ammonia nitrogen emanating from the rumen (Blouin et al., 1995). Saliva also contributes N to the rumen to assist in the support of microbial protein synthesis. Blood urea nitrogen is sometimes used as an indicator of excess soluble nitrogen. However, blood urea nitrogen levels can be high when there is also too much total protein in the diet. Excess escape protein will be deaminated, with urea the result. Therefore, using the potential for microbial protein synthesis, based on intake and considering positive and negative modifiers, is a useful guideline to evaluating the adequacy of the soluble nitrogen available.

The amino acid compositions of rumen bacteria and rumen protozoa are fairly consistent. In spite of considerable changes in diet and sampling time, Martin et al. (1996) found no differences in the amino acid composition of bacteria and protozoa measured at various times post feeding. Prestlokken and Harstad (2001) reported no effects of diet on the composition of bacteria and protozoa. Likewise, Rodriquez et al. (2000) found no differences in the profiles of bacteria that could be associated with level of intake. The composition of bacteria is, however, different than protozoa (Martin et al., 1996; Korhonen et al., 2002).

Of the microbial protein produced, approximately 70% will be in the form of amino acids (Korhonen et al., 2002). In other words, if 100 g of microbial protein are synthesized per kilogram of feed DM, then 70 will be in the amino acid form. Evans and Patterson (1985) used a mixture of bacteria and protozoa (80:20) to derive an average amino acid pattern for rumen microbes. The proportion was based on older data, suggesting that of the microbial protein entering the gut in dairy cows consuming high grain diets, 20 to 25% originated from protozoa and 75 to 80% from bacteria. The origin

of the profile used in the popular CPM model (O'Connor et al., 1993) is based on the pattern found for bacteria.

There are two ways of providing results of amino acid analyses. Some laboratories report results from amino acid analyses on a molar, rather than a weight basis. It is important to be able to distinguish the difference. Convention for feed ingredients is to express amino acid percentage on a weight and not a molar basis.

### *Escape protein*

Escape protein is the portion of the total feed protein that is not fermented in the rumen. Because each protein source is made up of several proteins with differing solubilities, the amino acid profile of the escape portion of the protein will differ somewhat from the native protein. Therefore, it is useful to be able to predict the profile for the escape portion. A good source of information is Dr. Peter Robinson's home page:

<http://animalscience.ucdavis.edu/faculty/robinson/default.htm>

### *Protected amino acids*

Protected amino acids have been available to the dairy industry for a considerable length of time. However, the addition of a protected amino acid will only be of benefit if it is limiting. The value of adding protected amino acids can be determined with the use of a ration formulating program specifically designed to address the issue of the amino acid needs of the dairy cow. Several amino acid products are readily available on the North American market (Table 2). A complete description of most of these has been provided by Lapierre et al. (2002). The potential advantage of being able to target amino acids is to meet a specific need and lower overage.

## Assessing the Needs of the Dairy Cow

Once some control over the supply can be exercised, it is important to be able to estimate the amounts that should be supplied to the cow. There must be an awareness of the performance potential to serve as a target, as well as knowledge of factors that impact this target. There must further be a procedure to assess temporary or long-term responses to shortages. Then, diets can be formulated to permit the cow to achieve her expected potential.

The lactating dairy cow needs amino acids for maintenance, growth, and reproduction, as well as for milk production. However, the amount needed for milk is generally much larger than the rest. O'Connor et al. (1993) and Evans and Patterson (1985) provided estimates of the needs for maintenance, growth, and production based on the efficiencies by which absorbed amino acids are used.

Studies of amino acid uptake to output ratios dating back as far as 1975 (Clark, 1975) have shown that total uptake by the mammary gland are consistently higher than output into milk for arginine, lysine, and the branch-chained amino acids. On the other hand, the mammary uptake of the non-essential amino acids, glutamate, aspartate, and proline is considerably less than the quantities in milk. Evans (1999) demonstrated that the uptake of total amino acids is similar to output, indicating that these particular essential amino acids are needed to synthesize the non-essential amino acids found in milk. Because of this, the pattern of amino acids needed for milk is different than the composition of milk (Table 3).

### *Amino acid ratios*

There is a certain amount of resilience built into the supply and demand within the udder. Practical formulation systems allow needs to be met to the extent possible, without elevating costs. Maintaining lysine and

methionine in a narrow range relationship to each other, especially without concern for the remaining essential amino acids, has the potential to elevate costs and provides no proven benefit. Although efficiency may be higher when a ratio is maintained between all amino acids, the ability to supply them in a particular ratio may pose difficulties. As a result, one or more amino acids will be over supplied. If one of the amino acids found to be in excess happens to be lysine or methionine, then in order to maintain a constant ratio, more of the other amino acid would be required. If needs are met, then further maintaining of a constant ratio will only result in additional wastage.

McGuire (1998) established that the mammary gland could alter the extent of extraction of nutrients based on the relationship between supply and need. This means that if there is an overage of one amino acid, there is no advantage to over formulating for another. Lacasse et al. (1996) demonstrated that secretory epithelial cells in proximity to arterioles produce nitric oxide from arginine. Nitric oxide is a vasorelaxant, increasing local blood flow. The mammary gland appears to have the flexibility to regulate its own supply of nutrients through a combination of altering blood flow rates and altering percentage extracted to meet the target requirement (Maas et al., 1998).

### *The need for non-essential amino acids*

Although systems, however crude, have been established to predict the utilization of essential amino acids, little regard has been given to the use of non-essential amino acids, and how they may impact the need for essential amino acids. It must be borne in mind that, if nonessential amino acids are not available, they will be synthesized from essential amino acids, much as occurs in the mammary gland. As well, Krebs's cycle intermediates contribute to the formation of non-essential amino acids.

The availability of non-essential amino acids may therefore be important in insuring the adequacy of the essential amino acid supply. Lobley et al. (2001) indicated that glutamine protects against methionine oxidation and that this amino acid can alter the need for methionine. As another example, glutamate is preferred over glucose as an energy substrate for mucosa cells. Stoll et al. (1999) determined that 94% of the enteral glutamate, but only 6% of the enteral glucose, was utilized by the gut mucosa. The gut requirement for glutamate may result in withdrawal of this amino acid from somatic tissues and the need to synthesize glutamate from arginine and proline.

The mammary gland and somatic tissues have the capability of synthesizing non-essential amino acids. However, it is important not to forget that the roles of these amino acids can be essential, and that as such, it may influence the supply of essential amino acids. It is advisable to provide total amino acids at levels that approximate the quantity needed for protein synthesis.

## Conclusions

Cows have amino acid requirements and respond to a change in supply. Both the supply of amino acids and the needs for amino acids can be estimated with reasonable confidence with the information currently available. Greater use of amino acid balancing programs would be instrumental in meeting performance potential. In addition, amino acid programs are helpful for whole farm nutrient management considerations and should be used to determine how to best meet the producers' goals.

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**Table 1.** Amino acid profiles of selected items (g/100 g amino acids).

Amino Acid	Rumen Bacteria <sup>a</sup>	Rumen Protozoa <sup>a</sup>	Soybean Meal <sup>b</sup>	Distillers Grains <sup>b</sup>	Gluten Meal <sup>b</sup>	Casein <sup>b</sup>
Arginine	4.8	4.6	7.3	3.7	3.0	3.7
Histidine	2.1	1.8	2.6	2.2	1.9	3.1
Isoleucine	5.2	6.0	4.5	3.5	3.9	5.2
Leucine	8.2	8.1	7.6	10.4	16.0	9.6
Lysine	7.1	10.2	6.0	2.3	1.6	8.1
Methionine	2.0	1.7	1.3	1.8	2.4	3.0
Phenylalanine	5.5	5.5	5.1	4.6	6.1	5.2
Threonine	5.8	5.6	3.6	3.5	5.3	4.2
Valine	5.6	5.3	4.8	4.8	4.4	6.6

<sup>a</sup>Korhoren et al., 2002.

<sup>b</sup>Degussa Corporation, 2001.

**Table 2.** Companies providing protected amino acids or amino acid analogs in North America.

Company	Amino Acid Provided
Adisseo	Methionine, methionine analog
Church and Dwight Co., Inc.	Methionine analog
Degussa Corporation	Methionine
Jefo Nutrition, Inc.	Methionine, lysine
Nisso, Inc.	Methionine
Novus International, Inc.	Methionine analog

**Table 3.** Uptake of amino acids by the mammary gland as compared to output in milk (g/100 g amino acids).<sup>a</sup>

Amino Acid	Total Uptake	Mammary Output
Arginine	8.53	3.40
Histidine	3.29	2.74
Isoleucine	8.80	5.79
Leucine	13.04	9.18
Lysine	9.14	3.40
Methionine	2.82	2.71
Phenylalanine	4.51	4.75
Threonine	4.76	3.72
Valine	10.01	5.89

<sup>a</sup> Evans, 1999