# Fermentrics™ Interpretation and Guidelines

Fermentrics™ is a novel laboratory method utilizing a batch-culture, rumen-fluid, gas-fermentation system combined with mathematical curve-peeling techniques allowing for the differentiation of rapid and slowly-fermenting carbohydrate pools in individual feedstuffs or TMR samples. The rate and extent of organic matter degradation, employing hundreds of data points, can be determined with Fermentrics™ by monitoring gaseous fermentation products ($CO_2$, methane) of microbial metabolism in addition to $CO_2$ produced by the buffering of microbial produced short-chained fatty acids (SCFA, primarily propionate, acetate and butyrate). This allows for a direct approach to determining carbohydrate pool ($B_1$, $B_2$, $B_3$) digestion rates to more accurately populate feed libraries in newer ration-balancing software. Fermentrics™ reports incorporate traditional nutritional parameters with unique analytes such as direct measurement of microbial biomass production and a microbial approach to measuring soluble protein.

While gas-fermentation systems are quite popular among European researchers there are only a few research labs in North America with gas-fermentation capabilities and they are not capable of processing and handling the sample volume needed in a commercial offering. The desire to provide more dynamic and diagnostic nutritional tools led to an August, 2010 joint initiative between Dairyland Laboratories, Inc. and RFS Technologies to commercialize Fermentrics™ and make this cutting-edge analysis widely available to North American livestock producers and their nutritionists.

RFS Technologies™ is a full service agricultural testing and research laboratory located Ottawa, Canada who have spent decades researching and field-testing Fermentrics™ out of the frustration of not being able to use current analytical techniques to understand and manipulate the biological potential of the rumen. This is not to diminish the value of wet chemistry or NIR analyses, but rather to point out their static nature which does not provide the dynamic or diagnostic approach needed to generate both qualitative and quantitative information on the rate and extent of digestion in a practical and inexpensive manner. Fermentrics™ is based on research conducted at Cornell University, University of Kentucky, the University of California, the Rowett Research Institute, the University of Hohenheim and the DLO Institute for Animal Science and Health.

Fermentrics™ can provide a unique perspective on the dynamics of feedstuff digestion not available from standard analyses. This allows enhanced insight as to the direction of corrective action when animals are not performing to expectations or can be used as a benchmark when animals are exhibiting superior performance. The following information is intended to aid producers and nutritionists in an understanding the analytes presented on the Fermentrics™ report along with benchmarking statistics from 945 TMR and 175 corn silage North American samples analyzed via Dairyland Laboratories through August 2013. It should be noted that statistics include from both good and poorly performing TMR's and corn silages. The TMR target values are provided as guidelines and not specific to all feeding situations. Fermentrics™ is an integrative system so multiple analytes must be considered in relationship to each other.

## Sample Handling

Feedstuff or TMR samples are dried overnight at a maximum $62^0$C, ground to 6-mm and sub-sampled for analysis. One portion is ground to 1-mm for the more traditional analyses provided on the report. The other portion of the 6-mm ground sample is sent to RFS Technologies™ for gas-fermentation analysis (two replicates/sample). Rumen fluid is collected from lactating cows producing a minimum of 65 lbs milk in a commercial dairy close to the lab. Cows are milked 2X/day and their diet is over 60% forage, starch levels over 20%, with no BST usage. Fluid is pooled from the donor cows. No dry or low production cows are used as donors. Cows are fed daily at 6:45am and fluid is collected at 9:30am. The rumen fluid is filtered through cheese cloth prior to addition of the buffer and fermentation runs begins roughly 30 minutes after collection. Standards are also incubated to monitor incubation consistency.

## Graph Interpretations

**X-axis** is time in hours.

**Y-axis** is ml of gas produced from the 400 mg sample.

**Total** represents the curve of the total amount of gas produced from a 400mg sample incubated in rumen fluid for 48 hours. In general, 60% of the total gas production asymptote is reached in the first 24-hours so this is the most important portion of the gas production curves to focus attention.

**Slow** represents the amount of gas produced from the slow pool. Given the heterogeneity of its nature, the slow pool is comprised primarily of fiber ($B_3$ Pool, hemicelluloses and cellulose) along with slowly degradable starch.

**Fast** represents the gas produced from fast pool consisting primarily of $B_1$ (starch) and $B_2$ (soluble fiber) pools; although it is possible for very rapidly digesting ($B_3$) fiber to also contribute to the fast pool of gas. In TMR's, fast pool gas amounts higher than about 40 ml of gas are indicative of excess acetate (which produces gas) and reduced levels of propionate (minimal gas production and more ATP) coming from the fast pool nutrients. TMR samples with over 40ml of gas from the fast pool generally appear to need more energy for microbial protein production, thus supplementation should be considered using propionate-producing feeds (grains) as opposed to forages or non-forage fiber sources (NFFS) like soy hulls or beet pulp.

It should be noted that these pools are not homogeneous because there can be both slow and fast pools within each carbohydrate fraction (e.g. the slow pool may contain some slowly digested starch). This fact may present challenges for those looking for an analysis that reflects the fermentation of chemically identifiable and measurable feed fractions, however, it does approximate the nature of ruminal fermentation and provides a practical means to evaluate rations, predict the productive response and make sound nutrition decisions affecting productivity and profitability (Johnston and Tricarico, 2007).

## Fast Pool Kd/hr

The fast pool rate (Kd) is derived from the maximum rate of degradation per hour of silage acids, sugar, rapidly degradable starch, and soluble fiber. Pell and Schofield (1993) published research on computerized gas production using individual vessel pressure sensors to relay gas pressure data. A more controlled system in conjunction with digestion kinetics analyzed by two-pool logistic models (curve-peeling) techniques (Schofield et al., 1994) further allowed gas fermentation to be divided into a "fast pool" (primarily $B_1$-starch and $B_2$-soluble fiber) and a "slow pool"(primarily $B_3$-insoluble available fiber).

## Slow Pool Kd/hr

The slow pool rate (Kd) is derived from the maximum rate of degradation per hour of the more slowly degraded $B_3$ (NDF) fiber pool (hemicellulose, cellulose) and due to its heterogeneous nature, may also include slowly degraded starch.

### C:B1, C:B2 and C:B3 Kd (%/hour)

Carbohydrate pool specific digestion rates (Kd) are the calculated maximum rates of degradation per hour for the $B_1$, $B_2$ and $B_3$ carbohydrate pools as defined by models like CNCPS or CPM. It should be recognized that strict definitions of B-pool constituents (e.g. $B_1$ is only starch) cannot be adhered to with this type of analytical tool given the heterogeneous nature of nutrients which can exist in both the fast and slow pools. However, Fermentrics™ B-pool rate estimates do allow nutritionists more realistic values than the "book values" contained in feed libraries or NDF digestion rates calculated from NDF, lignin and single time-point NDFD. Fermentrics™ captures over 5,000 gas data points in the 48-hour incubation. This allows curve-peeling software to detect fast pool and slow pool terminal rates from the inflection points in the total gas curve. Digestion rates are reported as normalized specific rates and not actual rates, therefore, you can have a high number if you have a small pool and a fast actual rate. Specific rates equal the terminal rate divided by the pool size (e.g. the gas produced by each pool). Large pools with the same actual rate will have smaller specific rates. See example:

| Actual rate (ml/hr) | Pool Size (e.g. max amt of gas produced) | Specific Rate (%/hr) |
|---|---|---|
| 10 | 50 | 20% |
| 10 | 100 | 10% |

Field experience suggests that $B_3$ (NDF) pool rates of less than 5% per hour are reflective of low digestibility forages resulting in reduced energy intake and microbial protein production. Depending upon the gas volume produced by the fast pool, increasing the supply of NFFS may be a solution to drive more production (see more detail in section entitled "Relative Proportion of Pools").

Chai et al., (2003) published equations for starchy feed ingredients and corn silage (Not TMR's) describing the relationship between gas levels and measured starch degradation. This allows for redefining the fast pool into $B_1$ (starch) and $B_2$ (soluble fiber) for better defining feedstuff kinetics in ration-balancing software.

Field experience suggests that $B_1$ pool rates in excess of 25%/hour are indicative of situations where the potential for ruminal acidosis, fat/protein inversions and poor hoof health exists. Rates that are slower

could be reflective of feeding dry corn versus fermented corn and can be lower yet in the rumen if processing of kernels in the corn silage is poor, or if the grain is not adequately processed (700-1200 microns; low end for dry corn and higher end for HMC). Fermentrics samples are ground to 6-mm so the starch particles will be very fine and particle size of on-farm feedstuffs can further reduce fast pool rates.

| TMR | Mean | Mean -/+ 2SD | Standard Deviation | Target |
|---|---|---|---|---|
| **C:B1 (starch) Kd** (maximum % \hour) | 17.9 | 8.9 – 26.9 | 4.5 | **18-20** |
| **C:B3 (NDF) Kd** (maximum %/hour) | 4.6 | 2.4 – 6.8 | 1.1 | **5-6** |
| **Corn Silage** | **Mean** | **Mean -/+ 2SD** | **Standard Deviation** | |
| **C:B1 (starch) Kd** (maximum % \hour) | 16.8 | 7.0 – 26.6 | 4.9 | |
| **C:B3 (NDF) Kd** (maximum %/hour) | 4.7 | 2.5 – 6.9 | 1.1 | |

## aPartitioning Factor

Apparent partitioning factor is a good indicator of the fermentation efficiency and is calculated as: (short chained VFA yield + microbial protein production)/total gas production. A higher aPartitioning Factor value indicate less gas and more ATP for increased microbial growth. As a general rule, higher levels of gas production indicate higher levels of acetate production which in turn results in lower ATP production.

The main VFA's produced during the fermentation are propionate, acetate, and butyrate. These VFA's produce different levels of ATP (energy) per mole; propionate 3, acetate 2, and butyrate 2. They are also associated with different levels of gas production with high acetate fermentations producing more gas (methane and C02) compared to high propionate fermentations. Blummel et al., (1997) published research on the relationship between gas from the production of SCFA and microbial biomass yield. He found an inverse relationship between gas production and microbial biomass yield when the variables were related to a given unit of truly degraded substrate. This is due to higher gas production when SCFA like acetate are produced compared to the ATP energy available for microbial growth when propionate is produced. Blummel proposed the concept of a partitioning factor (PF) which is the ratio of truly degraded substrate to gas volume produced. Interestingly, forages with a high PF (e.g. low gas production per unit of truly degraded substrate) exhibited higher intakes. Their dry matter intake prediction model included rate and extent of 24-hour gas production along with PF and accounted for 84% of the variation in the intake of fifty-four forages in their research.

TMRs that produce a high partitioning factor (>4) are strongly associated with higher propionate fermentations which produce more ATP and support increased microbial protein production.

| TMR | Mean | Mean -/+ 2SD | Standard Deviation | Target |
|---|---|---|---|---|
| **aPartitioning Factor** | 4.1 | 2.5 – 5.7 | 0.80 | **>4** |
| **Corn Silage** | **Mean** | **Mean -/+ 2SD** | **Standard Deviation** | |
| **aPartitioning Factor** | 3.7 | 2.0 – 5.4 | 0.85 | |

## aOMD – apparent organic matter digestibility

Apparent organic matter digestibility is the percent of organic matter digested. This can sometimes appear higher than what may occur in the animal because the Fermentrics™ batch system is designed not to run out of protein. If the soluble protein levels are low in the TMR, aOMD may be even lower in the animal than indicated on the Fermentrics™ report.

aOMD can be high but microbial biomass production low from too much acetate (which produces gas) and not as much propionate (more ATP for bacterial growth). The 2-pool total gas production is highly correlated with aOMD with a higher 2-pool gas total typically yielding a higher aOMD value, however, the relationship between gas production and aOMD must be considered. For example, a diet could have a very large slow pool (ml of gas from slow pool) yet exhibit a very slow rate (C:B3 Kd) with the majority of gas produced after 30-hours. This diet would likely predict a reasonably high aOMD but the nutritional contribution in a high-producing cow diet (with rapid rumen turnover rate) would be questionable due to the extended length of the fermentation.

| TMR | Mean | Mean -/+ 2SD | Standard Deviation |
|---|---|---|---|
| **aOMD (%)** | 60.5 | 46.1 – 74.9 | 7.2 |
| **Corn Silage** | **Mean** | **Mean -/+ 2SD** | **Standard Deviation** |
| **aOMD (%)** | 51.6 | 38.2 – 65.0 | 6.7 |

| aOMD % TMR Interpretation | |
|---|---|
| **<50%** | Milk production typically poor and formulating diets more of a challenge. |
| **~60%** | Milk production reasonable and formulating less complicated |
| **>65%** | Milk production good but attention to digestion rates and relative pool sizes is important |

## SP (BB) as a %CP

Soluble protein by buffer-borate (BB) method (Roe et al., 1990) is the traditional wet-chemistry approach to determining feedstuff soluble protein. There is some research that suggests this analysis may overestimate the amount of soluble protein utilized by rumen microbes (Reynal et al., 2007).

| TMR | Mean | Mean -/+ 2SD | Standard Deviation |
|---|---|---|---|
| **SP (Buffer-Borate) %CP** | 40.7 | 26.7 – 54.7 | 7.0 |
| **Corn Silage** | **Mean** | **Mean -/+ 2SD** | **Standard Deviation** |
| **SP (Buffer-Borate) %CP** | 54.5 | 33.7 – 75.3 | 10.4 |

## SP (Microbial) as a %CP

Soluble protein by microbial analysis is the amount of crude protein degraded in 3 hours of sample incubation divided by the total crude protein of the sample. If SP levels are below target, there is potential for excessive energy-spilling by rumen bacteria (Russell, 2002) which limits their growth. Correcting SP levels in the diet can result in increased microbial protein production and improved aOMD.

| TMR | Mean | Mean -/+ 2SD | Standard Deviation | Target |
|---|---|---|---|---|
| **SP (Microbial) %CP** | 40.7 | 22.9 – 58.5 | 8.9 | **35-38** |
| **Corn Silage** | **Mean** | **Mean -/+ 2SD** | **Standard Deviation** | |
| **SP (Microbial) %CP** | 51.9 | 28.5 – 75.3 | 11.7 | |

## Microbial Biomass Production (mg/g)

Microbial biomass production (MBP) is measured directly by analyzing the substrate that remains after 48 hour incubation with a NDF analysis (w/o amylase or sodium sulfite). The difference between the weight of the substrate before and after NDF analysis is the microbial biomass. Early versions of Fermentrics™ quantified VFA's and gas production, and then used a stoichiometric equation (Blummel et al., 1997) to predict the microbial biomass produced during the fermentation.

Higher MBP is somewhat the "gold standard parameter" associated with higher milk production. If the dry matter intake (DMI) of the diet is known, it is possible to convert MBP to estimated grams of rumen microbial protein produced by using this equation: MBP x 0.41 x 1.3 x Kg of DMI. The 0.41 is the assumed amount of microbial protein contained in the biomass being measured, 1.3 is an adjustment factor accounting for about 30% of the rumen bacteria existing in the liquid phase thus not measured in the biomass value. Using an actual TMR example with 160 mg/g MBP and an average cow DMI of 23.5 kg, equates to 2004 grams of microbial protein produced (Ipharraguerre and Clark, 2005). Total dietary microbial protein in grams divided by 70 equals the liters of milk potential from a protein perspective. The total contribution of microbial protein plus any RUP provided in the diet is what will contribute to the total protein supply utilized for milk production.

| TMR | Mean | Mean -/+ 2SD | Standard Deviation | Target |
|---|---|---|---|---|
| **Microbial Biomass Production** | 135.8 | 76.0 – 195.6 | 29.9 | **>140** |
| **Corn Silage** | **Mean** | **Mean -/+ 2SD** | **Standard Deviation** | |
| **Microbial Biomass Production** | 116.3 | 59.5 – 173.1 | 28.4 | |

## Relative Proportions of Pools

Fermentrics™ analyzes fermentation patterns over a 48-hour period thus there is no single metric that should be chosen as the measure of whether or not a diet will support high production levels. The 2-pool total gas production is highly correlated with aOMD with a higher 2-pool total typically yielding a higher aOMD value, however, the relationship between gas production and aOMD must be considered. For example, a diet could have a very large slow pool (ml of gas from slow pool) yet exhibit a very slow rate (C:B3 Kd) with the majority of gas produced after 30-hours. This diet would likely predict a reasonably high aOMD but the nutritional contribution in a high-producing cow diet (with rapid rumen turnover rate) would be questionable due to the timing of the fermentation. Other diets with equal aOMD estimates can have fermentations with very rapid rates that present a different set of challenges and dietary corrective actions needed to promote a rumen environment conducive to high production.

The amount and relative proportions of the ml of gas produced by each pool can help characterize the fermentation. Gas is generated by buffering the acids produced by rumen bacteria and from fiber fermentation gases (e.g. methane). When the fast pool size exceeds about 40-ml gas production, the higher gas production is likely from the rapidly digesting $B_2$ (soluble fiber) pool which produce considerable methane and carbon dioxide gas (along with acetate and less ATP for microbial growth) rather than from excessively available starch. In TMR's displaying a relatively slow, "slow pool digestion rate", there may be a tendency to consider the addition of NFFS sources. However, this would result in the production of more gas and not drive the fermentation towards desirable energy from propionate (grain; whose pathway does not produce gas) needed to support higher milk production. Diets with a large total volume of gas (e.g. >110 ml) may indicate excellent forage digestibility but lack suitable energy for ATP and microbial production due to bacterial energy-spilling (lack of SP) or lack of propionate-precursors (grain).

| TMR | Mean | Mean -/+ 2SD | Standard Deviation | Target |
|---|---|---|---|---|
| **Fast Pool** (ml of gas) | 29.5 | 16.3 – 42.7 | 6.6 | **<40** |
| **Slow Pool** (ml of gas) | 40.9 | 23.1 – 58.7 | 8.9 | **~60** |
| **2-Pool Total** (ml of gas) | 70.5 | 42.1 – 98.9 | 14.2 | **80-110** |
| **Corn Silage** | **Mean** | **Mean -/+ 2SD** | **Standard Deviation** | |
| **Fast Pool** (ml of gas) | 28.4 | 15.2 – 41.6 | 6.6 | |
| **Slow Pool** (ml of gas) | 40.0 | 20.2– 59.8 | 9.9 | |
| **2-Pool Total** (ml of gas) | 68.5 | 38.9 – 98.1 | 14.8 | |

## Relative Times to Max Rate

The relative times for each pool to reach its maximum rate of degradation is important to maintain synchrony of ruminal digestion. Field experience suggests that when the difference between "time to max" for the fast and slow pools exceeds 10-hours, poor production and/or lower components are likely to result. A wide difference may indicate the need for a more intermediate source of energy, such as dry corn (to slow down the fast pool Kd) or more corn silage and/or higher quality alfalfa/grass (to increase Kd of slow pool).

| TMR | Mean | Mean -/+ 2SD | Standard Deviation | Target |
|---|---|---|---|---|
| **Difference Between Slow Pool Time to Max Rate and Fast Pool Time to Max Rate** | 9.2 | 2.2 – 16.2 | 3.5 | **<10** |
| **Corn Silage** | **Mean** | **Mean -/+ 2SD** | **Standard Deviation** | |
| **Difference Between Slow Pool Time to Max Rate and Fast Pool Time to Max Rate** | 10.5 | 4.5 – 16.5 | 3.0 | |

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# Appendix A: Four Quadrant TMR Guidelines

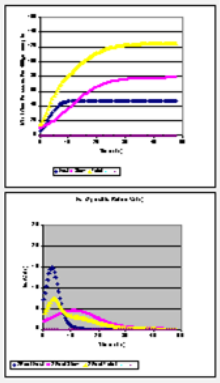
## QUADRANT I

Quadrant (and Kd's) where cows are typically milking well, but watch for any sudden changes in feed delivery or consistency

**Symptoms**
- Good manure consistency
- High dry matter intakes (DMI)
- Excellent milk yield
- Exceptional feed conversion ~ 1.7

**TMR Rates**
CB1 Kd: 18 – 20%/hr
CB3 Kd: 5 - 6%/hr

**Consider**
- Ensure enough NPN and peptides to feed rumen bacteria.



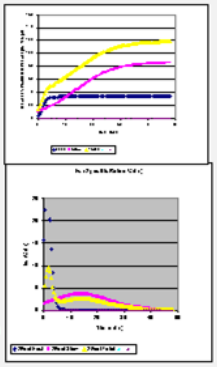
## QUADRANT II

Not enough energy

**Symptoms**
- Grain in manure
- Low milk protein
- Milk yield lower than expected
- Rough hair coats
- Poor reproduction

**TMR Rates**
Small pool of CB1 that disappears quickly
CB3 Kd: 5 - 6%/hr

**Consider**
- Increasing supply of rumen available starch and sugar (get B1 rates closer to 20-22%)
- Grinding grains finer
- Ensure adequate supply of peptide & NPN protein



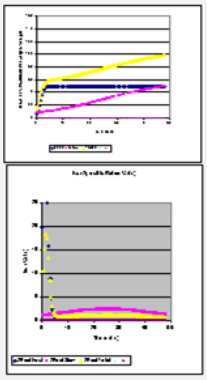
## QUADRANT III

Where the majority of "problem" herds reside. High acidosis potential due to a fast "fast pool" and a slow "slow pool"

**Symptoms**
- Sore Feet - Repro problems - Poor DMI
- Ketosis in early lactation cows
- Fiber in Manure - Low rumen pH
- Variability in body condition score
- Fat/Protein Inversion in >10% of the herd

**TMR Rates**
CB1 Kd: >25%/hr
CB3 Kd: <5%/hr

**Consider**
- Increase NDFD by adding non-forage fiber
- Beet Pulp, Brewers Grains, Soyhulls
- Slow B1 to <21%



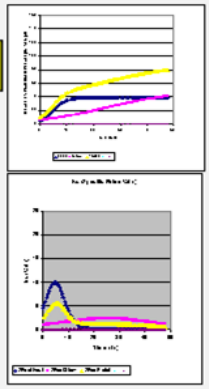
## QUADRANT IV

Slow "fast pool" and Slow "slow pool"

**Symptoms**
- Poor milk production
- Early peak/poor peak milk
- Cows "look" OK
- Diet CP is OK - but manure is stiff
- Average milk components
- Very expensive ration per CWT
- Reproduction- delayed, but not bad

**TMR Rates**
CB1 Kd: <15%/hr
CB3 Kd: <5%/hr

**Consider**
- Increasing rumen available starch and sugar
- Grind grains finer
- Increase NDFD by adding non forage fiber
- Add beet pulp, brewers, soyhulls, more digestible forages



# Appendix B: Fermentrics Report with Interpretative Guidelines