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VARIABILITY FACTORS AND USE OF MILK FAT-PROTEIN CONCENTRATION RATIO IN MICHIGAN HOLSTEIN DAIRY COWS

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VARIABILITY FACTORS AND USE OF MILK FAT-PROTEIN CONCENTRATION RATIO IN MICHIGAN HOLSTEIN DAIRY COWS

By

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ABSTRACT

VARIABILITY FACTORS AND USE OF MILK FAT-PROTEIN CONCENTRATION RATIO IN MICHIGAN HOLSTEIN DAIRY COWS

By

William Raphael

Descriptive statistics of and variability factors affecting the ratio of milk fat to milk protein concentrations (FPR) of 4359 Holstein cows from 68 Mid-western US herds were examined by the general-linear-models analysis. Peak mean FPR was 1.37 (SD = 0.33) and nadir mean FPR was 1.13 (SD = 0.22) and occurred at the first and tenth DHIA test days after calving, respectively. The proportion of cows with FPR < 1 ("inversions") was 8.6% at the first test day and reached a plateau at approximately 21% after test day four. A repeated measures, multivariate regression model identified test day number, herd, parity, season of calving, and peak milk production as significant variability factors. Proportion of variation due to herd was 44.0% and due to parity, season of calving, and peak milk production was not greater than 1% at any test day. Mean FPR, milk fat, and milk protein concentrations were calculated for strata of all variability factors and plotted against test day number. Data from the study population were used to develop a model by which FPR distribution of individual herds could be evaluated. This model adjusts test day FPR for known variability factors. FPR differences among herds identified by this model are probably explained by nutritional factors, so the model may be valuable for dairy nutrition problem-solving and monitoring.

DEDICATION

This thesis is dedicated to the world's dairy veterinary practitioners. These men and women are often faced with lack of reliable data to interpret the performance of their client's herds. This thesis will help fill this void.

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I am indebted to my committee members, Drs. Tom Herdt, Paul Bartlett, Michelle Kopcha, and Ron Erskine for their assistance throughout this research. Particularly, Dr. Tom Herdt, for his weekly meetings; also Dr. Leslie Fowler, for access to the herds she worked with, Joel Dobrzelewski, for his computer programming skills, Crystal Vierhout, for her technical assistance, and Drs. Rob Tempelman and Ted Ferris, from Michigan State University's Department of Animal Science, who brought new insight to the research.

I also acknowledge my family in Australia, who always keep me in touch with what the cows really need, and my fiancée, Jennifer Stuhler, for her love and support.

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KEY TO SYMBOLS OR ABBREVIATIONS

CLA = conjugated linoleic acid

DHIA = Dairy Herd Improvement Association

DIM = days in milk

DMI = dry matter intake

FPR = ratio of milk fat concentration to milk protein concentration from individual cows

MFD = milk fat depression

R^2 = coefficient of determination

INTRODUCTION

Dairy veterinarians are continually looking for new ways to monitor health and production. The DHIA records individual cow milk volume and composition on many farms. These are invaluable data. They have been used successfully for many years in the diagnosis and management of mastitis and for within-herd and among-herd comparisons of cows' production. New applications of these data have emerged, such as calculation of FPR, and the use of this ratio in assessment of herd health and nutrition. However, assessment of the distribution of FPR in North American herds is currently difficult because standards of comparison are not available. Similarly, there have been no reports identifying the variability factors affecting FPR in populations of commercial herds.

The objective of this thesis is to describe the distribution of FPR in Michigan dairy herds, and identify variability factors, excluding nutritional factors, affecting FPR. This will facilitate identification of abnormal FPR in cows and herds.

PREVIOUS WORK WITH FPR

The FPR has been examined to assess its usefulness in predicting energy balance, displaced abomasa, and fertility in dairy cattle.

There is an inverse relationship between FPR and energy balance. In an analysis of ten sets of data from four experiments conducted in the first three months of lactation, breed, parity, and ration adjusted correlation coefficients between FPR and energy balances were -0.36 to -0.74 ($P < 0.05$) (1). Regression analysis gave coefficients of -27.17 MJ to -52.46 MJ net energy for lactation per unit increase in FPR. Stobbs and Brett (1974) also report an important correlation between digestible organic matter intake and milk protein-fat concentration ratio ($r = 0.6473$, $P < 0.001$) (2). Additionally, Hagert (1991) calculated a correlation coefficient of $r = -0.68$ between FPR and energy supply (3).

The relationship between ketosis, a disease associated with negative energy balance, and FPR, further supports the relationship between energy balance and FPR. Heuer et al. (1999) calculated that the odds ratio for subsequent ketosis in cows with FPR > 1.5 at the first test day after calving was 3.2 ($P < 0.05$) relative to cows FPR $\leq$ 1.5 (4). Additionally, FPR > 1.5 was associated with subsequent loss of body condition (odds ratio = 1.8, $P < 0.01$).

Geishauser et al. (1998) conducted a case-control study in Ontario dairy herds to examine the FPR at the first DHIA test day after calving in cows that subsequently developed a displaced abomasum (5). Each case (n = 27) was matched by calving date and herd to three control cows. A FPR $\geq$ 1.39 was 8.2 times more likely (95% confidence interval = 2.7 to 25.0) to come from a cow subsequently diagnosed with a displaced

abomasum than a FPR < 1.39. In another case-control study, but this time examining herd differences, Geishauser (1996) compared 27-German-Friesian herds with displaced abomasa to 27-control herds, matched on milk production (6). Herds with displaced abomasa had increased FPR in the year prior to displacement being diagnosed than in herds with no displacements. No level of statistical significance is given and the source of the milk sample (individual cow or bulk milk tank) is not described. The authors in both of these reports speculate that displaced abomasa are preceded by negative energy balance and this is the reason why FPR can be predictive of displaced abomasa.

The relationship between FPR and fertility has also been researched. Heuer et al. (1999) calculated odds ratios for reproductive outcomes by FPR at the first DHIA test day after calving (4). Cows with FPR > 1.5 had an increased risk of cystic ovarian disease (odds ratio = 1.7, $P < 0.05$), reduced first service conception rate (odds ratio = 0.6, $P < 0.01$), five more days between calving and conception ($P < 0.05$), and 0.22 more services per conception ($P < 0.01$), relative to cows with FPR $\leq$ 1.5. In support of these findings, large (>0.4) increases in the FPR from the test day immediately before first insemination to the test day immediately after first insemination significantly reduced the first service pregnancy risk (odds ratio = 0.49, $P = 0.0002$) (7). All other positive changes in FPR and large negative changes (>-0.20) in FPR also significantly reduced the risk of pregnancy, although the odds ratios were closer to one (odds ratios = 0.77 to 0.87, $P \leq 0.0128$) (7). In contrast, Kristula et al. (1995) failed to demonstrate significance of first test day FPR in a multivariable Cox proportional hazards model identifying the variability factors affecting first insemination pregnancy rates, although it was significant in a bivariate Cox model analysis (8). Also, small correlation coefficients between 100-day and 305-day mean FPR

and four fertility traits (days from calving to first insemination, number of inseminations, number of days open, and 56-day non-return rate after first insemination) have been published (9). This is not surprising, considering how milk composition changes over the lactation.

It would seem that FPR can be a useful predictive tool to monitor herd health, particularly energy balance in recently calved cows, but also, possibly, individual diseases (ketosis, displaced abomasa) and infertility.

Interpretation of herd FPR data for nutritional and health monitoring is currently difficult because there has been no report, to our knowledge, of studies in North America directly examining the variability factors affecting FPR. There are several studies in which FPR was measured, although not as the primary objective, and these are discussed below. These reports provide some preliminary information about FPR variability factors.

Rodriquez et al. (1985) calculated seasonal influences on FPR (10). Prediction equations from the statistical analysis revealed that FPR increased at DHIA test days as maximum environmental temperature increased above 29°C. It also was increased as minimum environmental temperatures fell from 11°C to 0°C. These environmental influences on FPR were small but significant, accounting for 0.30% to 0.51% of the variability in FPR ($P < 0.05$). The effects of stage of lactation and stage of pregnancy on FPR were also small but significant, accounting for 0.3% and 0.2% of the variation respectively ($P < 0.05$). Prediction equations revealed that FPR decreased to the sixth month of lactation then increased. Sharma et al. (1990) reported similarly small variation in FPR attributable to stage of lactation and pregnancy (11). The trends observed from prediction equations were a decrease in the ratio from the beginning to 4.3 months of

lactation, followed by a small increase during the remainder of the lactation. Leoffler et al., 1999, found a small (r = -0.043) but significant ($P < 0.01$) negative correlation between FPR and DIM (7).

Heuer et al. (1999) reports that FPR at the first DHIA test day after calving varies significantly by parity ($P < 0.001$), being least in second parity cows (4). Additionally, these authors report strong, but small, herd effects on FPR and small correlation coefficients between milk yield and FPR at the first test day (r = -0.09).

EFFECTS OF RUMEN FERMENTATION ON MILK FAT CONCENTRATION

Understanding the variability factors affecting milk fat concentration is integral to a similar understanding of FPR. Feeding diets containing large proportions of concentrates commonly leads to MFD. The possible reasons for this cause of MFD are the focus of the following review.

The effect of feeding diets containing large proportions of concentrates (>50% dry matter, (12)) on milk fat concentration is consistently negative and large. As an example of the magnitude of MFD, Gaynor et al. (1995) classified cows as responders if they experienced a drop in milk fat concentration of at least one percentage unit when fed an 80% (dry matter) concentrate diet (13). The mean decrease in milk fat concentration for this group was 38% ($P = 0.0001$).

Trans-Fatty Acid Hypothesis

The specific biochemical changes that precede MFD are not known but experimental evidence suggests that dietary fatty acid supply and hydrogenation are important features.

Long-Chain Fatty Acid Hydrogenation in the Rumen

The majority of fatty acids present in ruminant diets are triglyceride esters of 18-carbon (C_{18}) chain fatty acids (14). Unsaturated forms of these undergo microbial hydrogenation in the rumen to become intermediary unsaturated fatty acids or stearic acid if hydrogenation is complete. Ruminant adipose tissue is composed of some fatty acids originating in the rumen. Adipose tissue composition changes once gastric fermentation

begins in the young ruminant and is a reflection of rumen microbial fatty acid hydrogenation. Demonstration of this is the increased depot fat concentration of stearic acid in a normal lamb compared to a gnotobiotic lamb (15).

The process of fatty acid saturation in the rumen occurs in steps. Intermediate products include various *cis*- and *trans*-fatty acid isomers with single or multiple double bonds. Their production is demonstrated by the presence of *trans*-fatty acids in ruminant adipose tissue. Experimental evidence of this is the more dilute concentration of *trans*-fatty acids in gnotobiotic lambs' depot fat compared to rumen-inoculated lambs (15). Under normal feeding practices, rumen biohydrogenation results in a characteristic pattern of positional isomers of $C_{18:1}$ where *trans*-11 comprises at least 80% (16).

Various factors have been identified which determine the fate of dietary fatty acids. Firstly, the mix of bacterial species within the rumen determines the extent of hydrogenation and end products formed. Sachan and Davis (1969) demonstrated that a *Borrelia sp.* hydrogenated linoleic acid to *trans*-11-octadecenoic acid (17). A *Pseudomonad* enzyme was shown to isomerize oleic acid to *trans*-10-octadecenoic acid (18). This reaction was pH dependent, the optimum pH being five. Strictly anaerobic bacteria, able to hydrogenate fatty acids, were isolated from sheep rumens (19). These were *Ruminococcus albus*, two *Eubacterium spp.*, and two *Fusocillus spp.* The *Fusocillus* organisms were able to hydrogenate oleic acid and linoleic acid to stearic acid, and linolenic acid to *cis*-15-octadecenoic acid. The others did not hydrogenate oleic acid but converted linoleic and linolenic acids to a mixture of octadecenoic acids; *trans*-11-octadecenoic acid predominated but several isomeric *cis*- and *trans*-octadecenoic acids were produced together with isomers of non-conjugated octadecenoic acids.

Secondly, the dietary supply of fatty acids also determines the extent of hydrogenation and end products formed. Harfoot et al. (1973) demonstrated that the fate of linoleic acid in *in vitro* rumen fluid incubations was dependent on the initial concentration of linoleic acid and availability of hydrogen donors (20). At concentrations of linoleic acid less than 1.0 mg/ml of rumen contents, stearic acid was the major end product. At concentrations greater than 1.0 mg/ml, the major end product of hydrogenation was the *trans*-11-monoenoic acid. Addition of sucrose as a hydrogen donor throughout the incubation period increased the extent of conversion to stearic acid. The authors implied that irreversible inhibition rather than competition for hydrogen donors was involved.

Thirdly, hydrogenation is pH dependent. *In vitro* rumen fermentation systems demonstrated that hydrogenation of oleic and linoleic acids to stearic acid is more complete at pH = 7.2 compared to pH = 6.2 (21). In support of this, Latham et al. (1972) demonstrated that hydrogenation of linolenic and linoleic acids from soybean oil was less in media from the rumens of cows fed diets containing large proportions of concentrates than diets containing large proportions of roughage, where pH is likely to be less (22). This was explained by reduced numbers of *Butyrivibrio fibrisolvens* and *Borrelia spp.* bacteria in the rumens of cattle fed diets containing large proportions of concentrates. Qiu et al. (2000) also demonstrated inhibition of biohydrogenation of linoleic acid *in vitro*, when pH was reduced from 6.5 to 5.8 (23). This conclusion was based on increased *cis*-9-*trans*-11-octadecadienoic acid, *trans*-$C_{18:1}$, oleic, and linoleic acid outflow and reduced stearic acid outflow from the more acidic *in vitro* fermentation systems compared to less acidic systems.

In summary, biohydrogenation in the rumen is likely to be less complete when the organisms capable of hydrogenation of dietary fatty acids are absent from the rumen, dietary fatty acids are concentrate in the rumen, and when rumen pH is relatively low.

Trans-Fatty Acids and Milk Fat Concentration Relationship

Davis and Brown (1969) made the first report of a suspected association between rumen fatty acid hydrogenation and MFD (24). Wide support for this theory has been received since then.

Milk fat content of *trans*-$C_{18:1}$ fatty acids is negatively associated with milk fat concentration. Griinari et al. (1998) report $R^2 = 0.82$ across 13 studies involving diets containing large proportions of concentrates (25). When four studies involving diets supplemented with unsaturated oils were added to the analysis, the R^2 was 0.54. In a study examining milk fat concentrations and milk fat *trans*-$C_{18:1}$ concentrations while feeding various oils, Wonsil et al. (1994) calculated an R^2 of 0.72 across 16 individual milk samples (26).

The effect of *trans*-$C_{18:1}$ fatty acids on milk fat concentration has been tested in cattle by feeding two diets that commonly result in MFD. These are diets containing large proportions of concentrates and diets supplemented with polyunsaturated oils. Both are thought to increase the flow of *trans*-$C_{18:1}$ fatty acids to the intestine.

Gaynor et al. (1995) conducted an experiment examining the effects of increasing the proportion of concentrate in the diet on the concentration of milk fat (13). Cows which experienced at least one unit decrease in milk fat percentage (responders) were compared to cows which experienced less than one unit decrease (non-responders). Responders were found to contain a significantly increased concentration of *trans*-$C_{18:1}$

fatty acids in their milk fat compared to non-responders. The correlation between the concentrations of *trans*-$C_{18:1}$ fatty acids in milk and the concentrations of fat in milk was r = –0.486 (P = 0.0001). Responders also had increased DMI and milk yield compared to non-responders, when fed the diet containing a large proportion of concentrates.

One of the earliest reports of an effect of dietary supplied *trans*-$C_{18:1}$ fatty acids on milk fat concentrations was conducted in mice by Teter et al. (1990) (27). Various fat supplemented diets were fed which differed in the ratio of *cis*-$C_{18:1}$-fatty acids to *trans*-$C_{18:1}$-fatty acids and in the concentration of linoleic acid. Diets with *trans*-$C_{18:1}$ fatty acids decreased the concentration of fat in milk. When lactating mice raised on *cis*-diets were crossed to *trans*-diets, the concentration of milk fat was decreased to concentrations similar to that of nursing females raised continuously on the *trans*-diets. Conversely, lactating females crossed from the *trans*-diets to the *cis*-diets produced milk with increased fat concentrations similar to mice fed the *cis*-diets continuously.

Wonsil et al. (1994) fed diets supplemented with various oils to cattle and found a significant negative association between the *trans*-$C_{18:1}$ flow in duodenal chyme and deviation in milk fat concentration, and also between *trans*-$C_{18:1}$ concentration in milk fat and deviation in milk fat concentration (26). Effects on DMI and milk production were variable.

Abomasal infusion of experimental treatments is performed to remove the rumen effects on dietary fatty acids. Abomasal infusion of *trans*-$C_{18:1}$-fatty acids resulted in markedly reduced milk fat concentrations and increased milk fat *trans*-$C_{18:1}$ concentrations relative to control and *cis*-$C_{18:1}$ infused groups (28). Abomasal infusion of

either fatty acid decreased DMI but did not affect milk production, relative to the control group.

In summary, the concentration of milk fat is decreased when *trans*-$C_{18:1}$ fatty acids are supplemented to the diet or when rumen production of *trans*-$C_{18:1}$ fatty acids increases, as occurs when diets containing small proportions of fiber are fed.

Additionally, the effects of the two dietary sources of *trans*-fatty acids are additive. In a 2 x 2 factorial experiment designed to test the effects of dietary fat (unsaturated vs. saturated) and rumen fermentation (diets containing large proportions of concentrates vs. large proportions of fiber) on milk fat concentration, both unsaturated fat diets and diets containing large proportions of concentrates suppressed milk fat concentration ($P < 0.05$ and $P < 0.01$, respectively) (25). The interaction approached significance at $P < 0.1$. DMI and milk production were only reduced by the effect of large proportions of concentrates in the diet.

Specific Isomers Involved

Since the association between MFD and *trans*-$C_{18:1}$ fatty acids has been made, research has been conducted to identify which isomers are involved. Advances in gas chromatography have facilitated this.

Griinari et al. (1998) observed an interesting isomer effect in a study examining the interaction between rumen fermentation and dietary fat (25). Milk fat concentrations decreased 7% when the fiber content was reduced in a saturated fat diet compared to 26% when the fiber content was reduced in an unsaturated fat diet. The effect of fiber and fat were significant ($P < 0.01$ and $P < 0.05$ respectively). The interaction term approached significance ($P < 0.1$). *Trans*-11-$C_{18:1}$ milk fat concentration decreased as the fiber

content of the diet was decreased ($P < 0.10$). *Trans*-10-$C_{18:1}$ milk fat concentration increased significantly by reducing the fiber content in the diet ($P < 0.05$). A consistent increase in the milk fat concentration of *trans*-11 and *trans*-10 isomers of $C_{18:1}$ were observed when unsaturated fat was added. *Trans*-10-$C_{18:1}$ milk fat concentration increased proportionally more when unsaturated fat was added to diets containing large proportions of concentrates vs. diets containing large proportions of fiber (590% vs. 112%, interaction $P < 0.05$), which coincided with the larger drop in milk fat concentration. Additionally, Piperova et al. (2000) demonstrated MFD feeding a 75% concentrate diet without buffers (29). Cows fed this diet had similar *trans*-fatty acid proportions as *trans*-10 and *trans*-11-$C_{18:1}$ (25 to 30%). In contrast, cows fed diets containing larger proportions of forages, which did not experience MFD, had approximately three times the proportion of *trans*-11-$C_{18:1}$ (approximately 30%) compared to *trans*-10-$C_{18:1}$. Piperova et al. (2000) also observed that *trans*-10-$C_{18:1}$ was the predominant *trans*-monoene during MFD (approximately 60% total *trans*-fatty acids) when a 70% concentrate diet containing five percent soybean oil was fed, and that the proportion of *trans*-11-$C_{18:1}$ was decreased, relative to a 40% concentrate diet (30).

These findings led to the conclusions that specific isomers are likely involved in MFD and that these may vary for different diets and vary in the magnitude of depression they cause. *Trans*-10-$C_{18:1}$ may be involved specifically for diets containing large proportions of concentrates. Griinari et al. (1997) cites an unpublished analysis that illustrates the same association (31). Against this theory is the small R^2 when milk fat concentration was regressed on milk fat concentration of *trans*-10-$C_{18:1}$ ($R^2 = 0.27$, n = 10).

Conjugated Linoleic Acids

CLA have been incriminated in the pathogenesis of MFD. CLA refer to a mixture of positional and geometric isomers of linoleic acid ($C_{18:2}$) with conjugated double bonds. Reports which document dietary induced MFD and increased milk fat CLA concentrations (13, 25, 30) and MFD when CLA is infused abomasally (32-35) or intravenously (36) support the relationship. In contrast, Teter et al. (1990) failed to demonstrate an effect of CLA on milk fat concentration in mice in their convincing experiment examining the effect of *trans*- and *cis*-isomers of $C_{18:1}$ (27).

The isomer positions of the *trans*-double bonds in CLA have specific effects on milk fat concentration. Griinari et al. (1997) speculated that the *trans*-10, *cis*-12 isomer might preferentially cause MFD over other isomers (31). In support, there was a greater decrease in milk fat concentration when *trans*-10, *cis*-12-$C_{18:2}$ was infused into the abomasum compared to *cis*-9, *trans*-11-$C_{18:2}$ (32, 33). This difference was significant in the second trial and not reported in the first. Milk yield and DMI did not differ between treatments in either experiment. Milk fat concentration was decreased 43% relative to controls and milk fat concentration of *trans*-10, *cis*-12-$C_{18:2}$ increased ten times, in a dietary trial with cows fed five percent soybean oil and 70% concentrate rations (30). The milk fat concentration of *cis*-9, *trans*-11-$C_{18:2}$ was decreased during MFD.

In vitro support for the effect of *trans*-10, *cis*-12-$C_{18:2}$ comes from a bovine mammary cell-culture study, where ^{14}C-acetate incorporation into cellular lipids was inhibited by 60% when this isomer was added to the culture (37).

Biochemical Pathogenesis

It is not known how *trans*-C_{18} fatty acids decrease milk fat concentration. Milk fat concentrations of short- and medium-chain fatty acids (C_{10} to C_{16}) decrease when MFD takes place (13, 26, 28, 35). This indicates decreased synthesis of de-novo mammary fat. Griinari et al. (1998) report a decreased milk yield of short-chain fatty acids in diets containing large proportions of concentrates vs. diets containing large proportions of fiber, but no effect of diet on the milk fat concentration of the same fatty acids (25).

Hypotheses to explain decreased milk fat concentration at the cellular level are reduced mammary triacylglyceride synthesis secondary to reduced acyl-transferase activity (28), direct effects of the physical and chemical properties of *trans*-$C_{18:1}$ fatty acids (25), and decreased Δ-9-desaturase activity (35). Other hypotheses are listed by Romo et al. (1994) (14).

Insulin Hypothesis

An early theory for MFD involved the effects that propionate and serum insulin concentrations have on the distribution of fat precursors between the udder and adipose tissue. The hypothesis that diet could affect body lipid distribution was first postulated by McClymont and Vallance in 1962 (38) and has largely been discounted by hyperinsulinemic-euglycemic clamp studies (39, 40).

The rumen volatile fatty acid profile after fermentation of diets containing large proportions of concentrates is well established. The molar proportional concentration of propionic acid increases, and those of acetic and butyric acids decrease (41-48).

Experiments have been conducted to simultaneously monitor rumen propionic acid production and milk fat concentrations when diets containing large proportions of

concentrates are fed, and the association with these variables and serum glucose and insulin.

McCullough (1966) calculated that rumen propionic acid proportional concentration accounted for 84% of the observed variation in milk fat percentages recorded in 34 feeding trials (49). Jenny et al. (1974) demonstrated decreased ruminal acetate and increased ruminal propionate proportional concentrations, and increased serum glucose and serum insulin concentrations in mid lactation cows fed rations containing large proportions of concentrates, compared to cows fed a control diet (50). Serum insulin was significantly correlated with milk fat concentration ($r = -0.87$), ruminal acetate ($r = -0.87$), ruminal propionate ($r = 0.85$), and serum glucose ($r = 0.71$). Serum glucose was significantly correlated with milk fat concentration ($r = -0.77$), ruminal acetate ($r = -0.76$), and ruminal propionate ($r = 0.73$). Linear regressions of the effect of serum insulin and glucose and ruminal propionate on milk fat concentration showed that propionate was the most important independent factor ($R^2 = 82.8$, $P < 0.01$). Diets, in this study, containing large proportions of concentrates, had significant negative impacts on DMI. Jenny and Poland (1975) demonstrated increased postprandial serum glucose and insulin concentrations in cattle fed a ration containing a large proportion of concentrates compared to cows fed a control ration (51). The cattle fed the ration containing a large proportion of concentrates experienced a 25% to 50% decline in milk fat concentration. Rumen propionic acid concentrations were not measured. Hurtaud et al. (1993) infused a mix of volatile fatty acids or propionic acid, ruminally, in isoenergetic quantities and observed that propionic acid decreased milk fat concentration compared to the mixed infusion group (52). There was no significant difference in milk yield or DMI ($P \geq 0.10$).

Sutton et al. (1988) concluded that the severity of MFD caused by feeding diets containing large amounts of starch in two meals, daily, reflected the concentration of volatile fatty acid production into a short period after a meal (53). MFD was a result of simultaneous sharp increases in propionate production in the rumen and serum insulin concentration. The severity of MFD was reduced with more frequent feeding.

From these and other experiments it was hypothesized that diets which increased serum insulin led to the uptake of fat precursors by adipose tissue at the expense of the udder, and hence prevented the udder from meeting its usual requirements for fat synthesis.

Research has been conducted to investigate this hypothesis. Benson et al. (1969) and Askew et al. (1971) observed little change in mammary lipoprotein lipase and glyceride synthetase activity in cows when switched from normal rations to restricted roughage rations (54, 55). In contrast, marked increases in adipose lipoprotein lipase and glyceride synthetase enzyme activities were observed (54). Opstvedt et al. (1967) observed that the enzymatic capacity for fat synthesis in abdominal adipose tissue was increased in cows fed an all-concentrate ration compared to cows fed an all-hay ration (56). In contrast, no large differences in enzyme activities were observed among mammary samples from cows fed the same rations. In two separate experiments, Laarveld et al. (1981 and 1985) demonstrated the refractory nature of the mammary gland to the effects of insulin (57, 58). In the former report, the extraction of glucose was not altered significantly by insulin. In the later report, insulin did not appear to alter the extraction of acetate, B-hydroxybutyrate, and triglycerides (all important milk lipid precursors) in the mammary gland. Additionally, Benson et al. (1972) demonstrated

increased adipose tissue glyceride synthetase and lipoprotein lipase activities and simultaneous MFD when diets containing large proportions of concentrates (likely to increase serum insulin) were fed (59). Also, Vernon (1980) summarized, in an extensive review, that insulin is the principal adipose anti-lipolytic hormone in ruminants (60).

These experiments lend support to the hypothesis that diets which stimulate insulin release are likely to stimulate fat synthesis in adipose tissue and not in mammary tissue.

To study the effects of insulin on milk fat concentration, without the confounding effect of hypoglycemia, a hyperinsulinemic-euglycemic clamp technique was employed (39, 40). Both studies maintained the clamp for four-days, with no effect on milk fat yield. Milk fat concentration was unchanged in the first study ($P < 0.40$) and reduced in the second study ($P < 0.01$), but both these results are confounded by decreased feed intake and the second by increased milk yield. Additionally, the second study demonstrated changes in milk fat composition in direct contrast to those commonly seen in MFD. The milk fat contained an increased proportion of medium-chain fatty acids and decreased proportion of long-chain fatty acids. Additionally, Gaynor et al. (1995) failed to demonstrate differences in serum insulin concentrations among cows suffering from at least one unit decline in milk fat percentage and those experiencing less than one unit decline, when diets containing large proportions of concentrates were fed (13). These three studies cast serious doubt on the hypothesis that serum insulin is integral in the MFD seen in dairy cows when diets containing large proportions of concentrates are fed. This is not conclusive, however, since Mackle et al. (1999) observed significant

decreases in milk fat concentration ($P < 0.01$), increased milk yield ($P < 0.02$), and no change in DMI during another hyperinsulinemic-euglycemic clamp (61).

EFFECTS OF RUMEN FERMENTATION ON MILK PROTEIN CONCENTRATION

Since FPR is calculated from the milk fat and protein concentrations, it is important to understand, in addition to the review of milk fat previously presented, the variability factors affecting milk protein concentration.

Dietary factors that cause MFD also affect milk protein concentration. In contrast to the large and negative effects on milk fat concentration, feeding diets containing large proportions of concentrates or small proportions of fiber increases milk protein concentration to a small degree. In the experiment by Gaynor et al. (1995), cited previously for the large decreases in milk fat concentration when 80% (dry matter) concentrate diets were fed, milk protein concentration increased only 1.8% (P = 0.06) (13). Although the effect seems consistent and is well documented, the reason why it occurs is not.

Two reports calculated correlations between milk protein concentration and energy intake (MJ/day) across multiple studies (62, 63). Emery (1978) used 13 reports and 44 treatment groups in the calculation (62): Sporndly (1989) used 53 reports and 179 treatment groups (63). The correlation coefficients were similar (r = 0.42). Regression indicated that an increased energy intake of one megajoule metabolizable energy per day increased the percentage of milk protein by 0.0036 units and 0.003 units (R^2 = 0.17) respectively. Sporndly found that the proportion of roughage in the diet, which was negatively associated with milk protein concentration in a simple correlation, ceased to become significant when the effect of energy concentration in the diet was removed. Emery noted that the energy association seemed to be independent of the ratio of

concentrate to roughage. Milk yield simultaneously increased with the protein concentration in both datasets. These two studies concluded that increasing the daily energy intake would increase the concentration of milk protein.

Propionic Acid Effects

One hypothesis to explain this increase in milk protein concentration is the amino acid sparing effect of providing gluconeogenic precursor (in the form of propionate) when concentrates are fed in large quantities (64, 65).

Studies have been conducted to evaluate the effects of propionic acid on milk protein concentration. In support, Rook and Balch (1961) and Rook et al. (1965) demonstrated significant increases (up to eight percent) in the concentration of milk protein with rumen propionic acid infusion (66, 67). In contrast, no significant change in milk protein concentration and a quadratic response in milk protein yield were reported in a trial designed to test the effects on milk composition of various quantities of propionate infused into the rumen (68). Milk yield increased linearly as propionate was infused in increasing quantities. Also in support of the amino acid sparing effect was the increase in plasma concentrations of Ala and Gln with increasing propionate infusions in this trial, and decreased concentrations of Gly and branched-chain amino acids. The later possibly indicates decreased tissue mobilization (68). In further support, Seal and Parker (1996) measured elevated total amino acid, essential amino acid, and non-essential amino acid concentrations in arterial plasma, mesenteric venous, and portal venous plasma of steers, with ruminal infusion of propionic acid (69). In a Latin square trial investigating supplementary rumen volatile fatty acids and protein types fed to cows, Huhtanen et al. (1998) found increased milk and protein yields with propionic acid infusion, but no

significant changes in milk protein concentration (70). Propionate infusion tended to increase plasma concentrations of Met and Thr ($P < 0.10$) and decrease the concentration of Gly ($P < 0.05$). The later is hypothesized to reflect decreased muscle amino acid turnover and net improvement in protein status. In an experiment designed to explore the combined effects of the source of energy (propionic acid vs. volatile fatty acid mixtures) and protein amounts (abomasal casein) on milk composition, Hurtaud et al. (1993) failed to demonstrate changes in either yield or concentration of milk protein with rumen propionic acid infusion (52). Casse et al. (1994) demonstrated decreased splanchnic release of glucose and increased release of acetate and Ala with intramesenteric venous infusion of sodium propionate (71). This is evidence that the liver was sparing gluconeogenic amino acids, but the experiment failed to demonstrate changes in milk protein concentration.

The conclusion from these studies is that milk protein concentration may be increased by rumen infusion of propionic acid mixtures. However, the effect is inconsistent. The effect may be mediated by increased availability of circulating amino acids for mammary protein synthesis.

Experiments are confounded by energy intake and DMI (Table 1). This may explain why results differ.

Role of Insulin

It is apparent from recent experiments that insulin may be involved in the regulation of milk protein synthesis. In the same experiment that placed doubts on the role of insulin in milk fat synthesis, Griinari et al. (1997) observed increased milk protein concentration ($P < 0.05$) with a hyperinsulinemic-euglycemic clamp (72). The effect was

larger with simultaneous abomasal casein infusion, indicating that the response to insulin is limited by absorbed amino acids. Milk protein concentration increased 0.03 and 0.29 percentage units from the baseline period for the clamp and clamp-casein groups respectively. Milk yield was increased and feed intake reduced by casein and the clamp respectively. The clamp reduced plasma branched-chain and essential amino acid concentrations and plasma urea nitrogen; the later indicating decreased amino acid oxidation. Together these results indicate improved efficiency of amino acid use for milk protein synthesis with hyperinsulinemia.

In a similarly designed trial, but this time fortifying casein with branched-chain amino acids, a hyperinsulinemic-euglycemic clamp increased milk protein concentration and milk yield (61). Milk protein concentration increased 0.17 and 0.33 percentage units for the clamp and clamp-protein groups respectively, compared to the control group. Insulin treatments reduced the concentration of milk urea nitrogen and plasma urea nitrogen and essential and branched-chain amino acids. DMI was not affected in this study in either treatment group. In contrast, McGuire et al. (1995) showed only small increases in the milk protein concentration ($P < 0.09$) by the end of a four-day hyperinsulinemic-euglycemic clamp (39). There was no effect on milk yield and modest increases in the yield of milk protein ($P < 0.05$). They did, however, demonstrate significantly decreased plasma urea nitrogen ($P < 0.001$) and essential amino acid concentrations ($P < 0.01$).

These reports offer another explanation of why diets containing large proportions of concentrates, which are known to increase serum insulin (50, 53), also increase milk

protein concentration. Interestingly, the magnitude of the response in milk protein concentration in two out of three of these trials is large.

Microbial Crude Protein Effects

A theory which has received less attention in the literature relative to others is the possible association between milk protein concentration and increased duodenal flow of bacterial crude protein, when diets containing large proportions of concentrates are fed. Replication of rumen bacteria is known to be dependent on supply of, among many things, fermentable carbohydrate to the rumen (73). Poore et al. (1993) conducted an experiment to test the effects of rumen starch degradation on milk composition and yield using diets that differed in the rate of starch degradation (dry rolled or steam flaked sorghum (milo)) (74). Milk yield remained unchanged but milk protein concentration increased significantly ($P < 0.04$) with increased rumen starch degradability. DMI was similar between diets. The flow of bacterial nitrogen to the duodenum was greater on diets with more degradable starch ($P < 0.01$).

In an experiment designed to test and compare this theory with the amino acid sparing effect of propionate, cows were either infused with glucose ruminally or propionate duodenally (75). Total carbon infusion and DMI were similar between groups. Increased milk protein concentration ($P < 0.08$) and increased duodenal microbial crude protein flow ($P < 0.25$) were observed with ruminal glucose, compared to duodenal propionate. Milk yields were similar between the two groups.

It would seem that reasonable support exists for a positive relationship between milk protein concentration and microbial crude protein flow to the intestine of cows fed diets containing large proportions of concentrates.

OTHER FACTORS AFFECTING MILK COMPOSITION

The previous discussion focuses on the effect of feeding diets containing large proportions of concentrates and small proportions of forage based fiber on milk fat and protein concentrations.

There are other important factors that affect milk fat and protein concentrations, and so also affect FPR. These have been reviewed extensively by Crabtree, 1984, Emery, 1988, Sutton, 1989, Sutton and Morant, 1989, Palmquist et al., 1993, and Fredeen, 1996, among others (12, 64, 76-79). It is beyond the scope of this review to repeat these authors, but rather, list the major factors involved.

Milk Fat Concentration

The factors affecting milk fat concentration can be classified as either dietary or non-dietary. The predominant dietary influence has been the focus of the previous review, but a significant other dietary factor is the effect of dietary fat on milk fat concentration.

Dietary fat has a variable effect on milk fat concentration, depending on the saturation of the fatty acids fed and the effects on rumen fermentation. The negative effect that unsaturated fatty acids have on milk fat concentration is proposed to occur secondary to *trans*-fatty acid formation. Saturated and unsaturated fatty acids are equally capable of decreasing rumen fiber fermentation, especially when fed over six to eight percent dry matter (12), and this also leads to MFD. The mechanism by which this occurs has not been elucidated, but seems to be reduced if physical contact between the fat and fiber in the ration can be prevented (77). Perhaps associated with this is a known antimicrobial effect of dietary fat (79).

If the dietary fat can be protected from microbial actions so that *trans*-fatty acids are not synthesized or fed in a manner so that rumen fermentation is not impaired, then milk fat concentration may increase (12). Examples include feeding rumen-protected fat (12) and reducing fiber and fat physical interaction within the rumen by mixing the fat and roughage separately at feed preparation (77).

The effects of dietary fat on milk fat concentration are frequently confounded by the positive effects on milk yield (12, 64, 79).

Other factors which affect milk fat concentration are those which affect rumen pH and hence the biohydrogenation of dietary fatty acids. There are a myriad of such factors, but the most significant are the type of dietary fiber (77), the physical characteristics of forages (12, 77, 79), and dietary buffer supply (77). Rumen pH is elevated or maintained when rumination takes place or when dietary ingredients buffer fermentation acids. Rumination is effective because it stimulates flow of salivary bicarbonate to the rumen and also rumen mixing, and hence absorption of fermentation acids. Feeding various types of fibrous feeds facilitates this, such as long stem forages. By-product feedstuffs are also fibrous (e.g. distillers' grain) and when substituted for concentrates in the diet as a source of fermentable carbohydrate, help normalize milk fat concentrations (64).

There are several significant non-dietary factors that affect milk fat concentration. The most important are stage of lactation, energy balance, the cows' environment, and lactation number.

Reports of the effects of stage of lactation differ because of the effects of other non-dietary and dietary factors, but the gross effects are similar among reports. These are a decline in fat concentration to a nadir around the time of peak milk volume production,

then a gradual increase to the end of lactation. The nadir was reached at four-months of lactation in Holsteins (11). The peak fat percentage was noted at the commencement of lactation in Holsteins by Schutz et al. (1990) and the nadir at around 50 DIM (80). Rodriquez et al. (1985) quantified the variation in milk fat concentration attributable to stage of lactation at 3.7% for Holsteins, larger than the effects attributable to climate (2.9%) (10).

Negative energy balance is associated with increased milk fat concentrations (1, 2) and compositional changes reflecting decreased de-novo synthesis (reduced proportions of milk fat short-chain fatty acids and increased proportions of long-chain fatty acids) (2). In this report, the best correlation between digestible organic matter intake (100%, 75%, and 50% ad lib intake) and various production variables, including fat and protein percentages and milk yield, was with fatty acid composition of milk fat (C_4 to C_{16} r = 0.7317, $C_{18:1}$ r = -0.7429) and protein:fat ratio (r = 0.6473). These coefficients were much greater than blood non-esterified fatty acid concentration (r = -0.2451) and milk yield (r = 0.5670).

Milk fat concentration decreases in the summer (77). Milk fat concentration decreased as the DHIA test day maximum temperature increased from 9.4°C to 36.1°C and decreased as relative humidity increased to 80% (10). Month of calving also affects milk fat concentration. Schutz et al. (1990) report increased test day fat percentages in cows calving from April to August, in Minnesota, relative to other months (80). Crabtree (1984) and Emery (1988) both report decreased milk fat concentration with increasing parity, but the confounding effect of increased milk production is not clear (76, 77).

There is a negative correlation between milk volume and milk fat concentration (81) although this is frequently measured as a stage of lactation effect (10, 11).

Milk Protein Concentration

An important dietary factor decreasing milk protein concentration is lipid (12, 64, 65, 79, 82). This effect is often confounded by increased milk yield (12, 64, 65, 79, 82). The mechanism of action has not been elucidated (64).

Milk protein concentration varies with stage of lactation, declining rapidly to a nadir at five to ten weeks after calving and increasing slowly from then until the end of lactation (11, 76, 80, 82, 83). Protein concentrations are increased in the first seven days of lactation, relative to later lactation, largely reflecting increased milk globulin concentrations (76).

There is a direct relationship between milk protein concentration and energy balance. Stobbs and Brett (1974) report a decline in milk protein concentration from 4.01% to 3.67% with a reduction in feed intake from ad lib to 50% of ad lib quantities (2). Grieve et al. (1986) calculated the correlation coefficient between milk protein concentration and energy balance to be $r = 0.12$ to $r = 0.47$, depending on the dataset (1).

Climate also influences milk protein concentration. Although the effect of stage of lactation was greater in magnitude, the combined effect of DHIA test day maximum and minimum temperatures and relative humidity was significant, accounting for 6.33% of the variability in Holsteins' milk protein concentration (10). Stage of lactation accounted for 9.3% of the variability. Milk protein concentration decreased as the maximum temperature increased from 9.4°C to 36.1°C and decreased as relative humidity increased to 80%. Ng-Kwai-Hang et al. (1982) concluded that seasonal variation may be ill defined

once stage of lactation, age of cow, and milk somatic cell score are accounted for (83). However, in a review by DePeters and Cant (1992) it is concluded that hot environmental temperatures reduce total protein content of milk (82). The reports by Schutz et al. (1990) and Crabtree (1984) are in direct contrast to this, although the former is an analysis of the effect of season of calving (not season of test day) and the later is based on bulk tank milk analyses (76, 80).

The protein content of milk shows a small decline with increasing age (76, 83). Lactation curves from Mid-western United States' Holsteins indicate that milk protein concentration varies very little amongst parities early in lactation, but after 225 DIM, shows disparity (80). It is greatest in second lactation cows and least in first lactation cows. There is a negative correlation between milk volume and milk protein concentration (81) although this is frequently measured as a stage of lactation effect (10, 11).

CONCLUSIONS

The FPR is likely to be influenced by all factors affecting the milk components individually. Though many of these are nutritional factors, non-nutritional factors also affect milk composition. The purpose of this thesis is to 1) identify these non-nutritional variability factors affecting FPR and 2) describe how clients' FPR data can be compared among each other, after adjustment for known significant variability factors.

CHAPTER ONE

VARIABILITY FACTORS AFFECTING MILK FAT-PROTEIN CONCENTRATION RATIO IN MICHIGAN HOLSTEIN DAIRY HERDS

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ABSTRACT

The objective of this report is to examine distribution of and variability factors affecting the ratio of milk fat concentration to protein concentration (FPR) in Holstein cows in 68 Michigan commercial herds. Peak mean FPR was 1.37 (SD = 0.33) and occurred at the first DHIA test day after calving. Nadir mean FPR was 1.13 (SD = 0.22) and occurred at the tenth test day after calving. The proportion of cows with FPR < 1 (inversions) was 8.6% at the first test day and reached a plateau at approximately 21% after test day four. In a repeated measures multivariable regression model with FPR at the first ten test days as the dependent variables, FPR varied significantly by herd, parity, season of calving, peak milk production, and test day number. The effect of peak milk production was quadratic. All main effects had a significant interaction with test day number. Proportion of variation due to herd was 44.0% and due to parity, season of calving, and peak milk production did not exceed 1%. Therefore, diagnostic assessment of a herd's FPR distribution must take days in milk, parity, season of calving, and peak milk production of cows into consideration, in addition to nutrition.

INTRODUCTION

The FPR may be a useful index of nutritional status and health. Several reports have examined the relationship between energy balance and FPR, with correlation coefficients in the range r = -0.36 to -0.74 ($P < 0.05$) (1, 2). This, and research indicating increased risk of subsequent ketosis (odds ratio = 3.2, $P < 0.05$) and increased subsequent body condition loss (odds ratio 1.8, $P < 0.01$) if FPR > 1.5 at the first test day after calving (4), suggest that FPR is a useful measure of energy balance in dairy cattle.

The FPR has also been demonstrated to be predictive of displaced abomasa, although with low sensitivity and specificity (80% and 68% respectively, FPR $\geq$ 1.39, odds ratio = 8.2, $P < 0.05$) (5). There are also reports indicating a negative relationship between FPR and fertility (4, 7, 8).

Interpretation of herd FPR data for nutritional and health monitoring is currently difficult because there has been no report, to our knowledge, of studies in North America examining the variability factors affecting FPR. There are several studies, as discussed below, in which FPR was measured, although not as the primary objective. These reports provide some preliminary information about FPR variability factors.

Rodriquez et al. (1985) calculated seasonal influences on FPR (10). Prediction equations from the statistical analysis revealed that FPR was increased at DHIA test days when maximum environmental temperature exceeded 29°C. It also was increased when minimum test day environmental temperature was in the range 0°C to 11°C. These environmental influences on FPR were small but significant, accounting for 0.30% to 0.51% of the variability in FPR ($P < 0.05$). The effects of stage of lactation and stage of pregnancy on FPR were also small but significant, accounting for 0.3% and 0.2% of the

variation respectively ($P < 0.05$). The FPR decreased from the start of lactation to the sixth month of lactation, then increased. Sharma et al. (1990) reported similarly small variation in FPR attributable to stage of lactation and pregnancy (11). The trends observed from prediction equations were a decrease in the ratio from the beginning to 4.3 months of lactation, followed by a small increase during the remainder of the lactation. In a study examining predictors of fertility, there was a small (r = -0.043) but significant ($P < 0.01$) negative correlation between FPR and DIM (7).

Heuer et al. (1999) reports that FPR at the first DHIA test day after calving varies significantly by parity ($P < 0.001$), being lowest in second parity cows (4). Additionally, these authors report strong, but small, herd effects on FPR and a small correlation coefficient between milk yield at the first test day and FPR at the first test day (r = -0.09).

The FPR is directly proportional to milk fat concentration and inversely proportional to milk protein concentration. Therefore, the variability factors affecting these milk components are likely to be also variability factors affecting FPR. Milk fat concentration is affected by diet, particularly diets containing large proportions of concentrates and containing unsaturated lipid or large quantities of any lipid (12, 13, 25). Milk protein concentration varies with level of energy intake (62, 63), dietary carbohydrate characteristics (13, 74), and dietary lipid content (12, 79). Both milk fat and milk protein concentrations vary by parity or age (76, 80, 83), stage of lactation (11, 76, 80), seasonal factors (10, 77, 82), and level of milk production (81).

The objective of this report is to describe the variability factors, excluding nutritional factors, affecting FPR in Michigan Holstein cows and discuss why these associations might exist.

MATERIALS AND METHODS

Data Collection

Production data from 89 herds that participated in a study measuring the prevalence and severity of lameness in Michigan Holstein herds were made available from Dairy Records Management Systems[1]. Herds used in that study were selected randomly from respondents (n = 388) to a descriptive letter sent to all DHIA subscribing herd owners within a 120-km radius of Lansing, Michigan (n = 612), but who were milking 50 to 300 cows.

Production data were gathered for lactations commencing between July 1, 1997 and June 30, 1998. If cows had calved more than once during this interval, the last lactation was selected. Milk was sampled repeatedly during these lactations by DHIA on occasions commonly referred to as "test days". Test days typically occur at monthly intervals, hence there usually is a maximum of ten test days in a 305-day lactation. Lactations with milk fat and protein concentration data for the first ten test days (test day one to test day ten) after calving were selected.

Since test day one is always the first DHIA test after calving, and test day ten, the tenth after calving, the test day number approximates stage of lactation. However, considerable variation in DIM among cows at each test day is expected, due to different time intervals between calving and test day one and different inter-test day time intervals. To minimize this variation during the stage of lactation when milk fat and protein concentrations change markedly, cows with DIM at the second or third test days outside of the 2.5^{th} to 90^{th} percentile range were excluded. The first test day was examined in a

similar fashion although the lower DIM limit was increased. Lactations with DIM at the first test day less than 7 days or greater than the 90th percentile were excluded. If the milk composition was estimated, rather than measured, on any test day, that entire lactation was deleted from the analysis. Examples of such test days are when milk samples become frozen or sour. Lactations were also excluded if any part of any test day sampling procedure was recorded as abnormal.

Fourteen herds were excluded because the data selection criteria resulted in them contributing less than 20 observations to the dataset. This reduced the final dataset to 4359 cows from 68 herds.

Statistical Analysis

A repeated measures, multivariable regression model was created, based on the significance of Type III sum of squares (84). The FPR at the first ten test days after calving (numbered one to ten) was the repeated, dependent variable. Independent variables considered were test day number, herd, parity, season of calving, and peak milk production. Parity was classified categorically at three levels (1, 2 or 3), as first, second, and third or greater lactation. Parity was also considered as a continuous variable and a categorical variable without grouping of parity three or greater. Season of calving was classified categorically at four levels; summer (June 21 to September 21), fall (September 22 to December 21), winter (December 22 to March 19), and spring (March 20 to June 20). Peak milk production was the greatest test day milk weight during the lactation. Linear and quadratic effects of peak milk production were evaluated. Peak milk production was also evaluated as a categorical variable at levels <36.3 kg, 36.3 kg to 45.3

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kg, 45.4 kg to 54.3 kg, and ≥54.4 kg. Interaction terms considered were parity x peak milk production, season of calving x peak milk production, and parity x season of calving.

The model was created by selecting independent variables in a manual, forward, step-wise manner, excluding variables with a level of significance greater than $P = 0.05$. Shapiro-Wilk tests of normality were performed on dependent variables and residuals from the model (84).

The probabilities of Type I error for between-subject effects in the multivariate model and also the R^2, probabilities of Type I error, and regression coefficients at each test day (univariate model analysis) were computed using the GLM procedure within SAS (84). The mixed procedure within SAS was also used, but abandoned because the computation exceeded the capability of desktop hardware (84). Proportion of total FPR variation due to each variable was approximated by calculating ω^2 (85). Probabilities of Type I error for within-subject effects in the multivariate model were computed using Greenhouse-Geisser adjustments of univariate tests (84) and multivariate analysis of variance. Tests for sphericity (transformed variates and orthogonal components) were computed (84). Specific contrasts were by the Scheffé method (84).

RESULTS

The FPR at each of the first ten test days are shown in Table 2. The median number of lactations per herd was 53 (range = 21 to 188). Frequencies of lactations in first, second, and third or greater parities were 1672, 1185, and 1502 respectively. Median parity number was four (range = three to eleven) in the parity three or greater class. Frequencies of lactations with season of calving as summer, fall, winter, and spring

were 1222, 1233, 928, and 976, respectively. Means of peak milk production in the entire data set, for parity classes 1, 2, and 3, and summer, fall, winter, and spring calving cows were 44.6 kg, 38.6 kg, 47.3 kg, 49.3 kg, 43.6 kg, 45.0 kg, 46.3 kg, and 43.9 kg, respectively. Standard deviations were 9.2 kg, 6.8 kg, 8.3 kg, 8.6 kg, 9.4 kg, 9.2 kg, 9.4 kg, and 8.8 kg, respectively. The proportion of first, second, and third or greater parity cows that had peak milk production on test days one to four was 47%, 81%, and 83%, respectively. The median DIM at each test day is described in Table 2.

The repeated measures, multivariable regression model includes the fixed effects of test day number, herd, parity, season of calving, and the parity x season of calving interaction, and also the linear and quadratic components of the peak milk production effect. Parity is classified categorically at three levels (1, 2 or 3), as first, second, and third or greater lactation, although regression coefficients for all other main effect variables did not change appreciably when parity was classified categorically at eleven levels or when parity was considered as a continuous variable. Peak milk production is a continuous variable in the model, although all other regression coefficients did not change appreciably when peak milk production was classified categorically.

Shapiro-Wilk tests indicated that FPR was normally distributed at all test days (W = 0.83 to 0.99). Residuals from the model were also normally distributed (W = 0.80 to 0.99).

The effect of herd ($P < 0.0001$), parity ($P < 0.0001$), season of calving ($P = 0.0091$), the linear ($P < 0.0001$) and quadratic terms ($P < 0.0001$) for peak milk production, and the interaction term for parity x season of calving ($P = 0.0264$) were all significant as between-subject effects in the multivariate model.

Tests for sphericity (transformed variates and orthogonal components) were significant ($P < 0.0001$) so the within-subject effects were determined from multivariate analysis of variance. However, this yielded similar probabilities of Type I errors to using Greenhouse-Geisser adjustments of univariate tests. The test day number ($P < 0.0001$), test day number x herd ($P < 0.0001$), test day number x parity ($P < 0.0001$), test day number x season of calving ($P < 0.0001$), and the linear ($P < 0.0001$) and quadratic ($P < 0.0001$) peak milk production x test day number interaction terms were significant. The interaction term for test day number x parity x season of calving was not significant ($P = 0.1736$).

All univariate models were significant ($P < 0.0001$). Coefficients of determination were highest at the second test day ($R^2 = 0.29$) and lowest at test day ten ($R^2 = 0.14$). The significant variables are described in Table 3. The proportion of total FPR variation due to herd factors was 44.0% in the multivariable model, and varied from 11.3% in the non-repeated measures model at test day ten to 25.7% at test day two. The proportion of variation due to parity, season of calving, and peak milk production did not exceed 1% at any test day. Regression coefficients are described in Table 4.

The proportion of test day FPR data with a value less than one (commonly referred to as "inversions") is illustrated in Figure 1. Individual herd mean and mean of herd mean FPR are plotted against test day number in Figure 2.

Parity effects are illustrated in Figure 3. First parity cows have a significantly lower FPR ($P < 0.05$) compared to the other parities for the first six test days. Second parity cows have significantly lower FPR ($P < 0.05$) compared to third or greater parity cows at the first two test days.

Season of calving effects are illustrated in Figure 4. Fall and winter calving cows had significantly higher FPR ($P < 0.05$) at the first test day compared to spring and summer calving cows. Winter calving cows had significantly higher FPR ($P < 0.05$) than spring, summer or fall calving cows at test days two and four to seven.

Peak milk production effects are illustrated in Figures 5 and 6. Cows with ≥54.4 kg peak milk production had significantly lower FPR ($P < 0.05$) compared to cows with peak milk production of 36.3 kg to 45.3 kg and 45.4 kg to 54.3 kg for all test days except the first and fifth (Figure 5). Cows with <36.3 kg peak milk production had significantly lower FPR ($P < 0.05$) compared to all other peak milk production groups at the first test day.

The parity, season of calving, and peak milk production variations in milk fat and protein concentrations are illustrated in Figures 7 to 12.

DISCUSSION

The data used in this report are from herds that were recruited into a separate study measuring the prevalence and severity of lameness in Michigan. The high response rate (63%) to the letter requesting expression of interest in that study and random selection of herds from respondents suggest that the effect of bias is likely to be minimal and that the herds selected for the study are representative of the Mid-western United States. However, it is possible that recruitment was more successful among herds experiencing lameness. This would affect inference of the results of this study, because of the association between lameness and nutritional circumstances that influence milk composition.

The significant change in FPR through the lactation ($P < 0.0001$) indicates that interpretation of herd FPR requires consideration of the distribution of DIM among cows within the herd. The prevalence of FPR values less than one ("inversions") is lowest early in lactation and plateaus after the fourth test day. It is possible that primary stage of lactation effects (11, 76, 80), negative energy balance (1, 2), or other nutritional factors, may be the cause of high FPR values in early lactation, through increased milk fat concentration. Possible nutritional factors influencing FPR are lipid feeding (12, 25) or high levels of forage in the diet (13, 25). Only the former is likely to be fed to cows in early lactation, which suggests that either primary stage of lactation effects, energy balance effects or dietary lipid are involved.

The individual herd FPR vs. test day plots, in Figure 2, being approximately parallel, indicate that between-herd differences are approximately uniform over the lactation. This indicates that there were few changes in within-herd factors such as nutrition and housing over the time period during which the research was conducted. However, a significant test day number x herd interaction ($P < 0.0001$) indicates that this is not so. A reasonable conclusion from this is that within-herd factors, such as nutrition, did change over the study period, but to a small degree.

Figure 2 also illustrates the significant difference among herds' mean FPR ($P < 0.0001$). The proportion of total variation of FPR due to herd factors is large (44.0%). The most likely explanation for this is the among-herd variation in nutrition, because it is an important determinant of milk composition and differs markedly among herds, although other herd factors, which could be responsible, are genetics and housing. The most likely nutritional factors are the proportions of the diet as concentrate, forage, and

lipid, and the characteristics of each of these ingredients. The authors of this report suggest using the distribution of values in Figure 2 to determine whether a herd of interest has extreme mean FPR.

The FPR increases with increasing parity number at the first six test days (Figure 3). This is because milk fat concentrations increase and milk protein concentrations decrease with increasing parity (Figures 7 and 8). These changes in milk composition suggest that a negative relationship between parity and energy balance may be the reason parity three or greater cows have increased FPR compared to other parities. Negative energy balance has previously been associated with increased milk fat concentration and FPR, and decreased milk protein concentration (1, 2). Alternatively, the parity effect seen here may be due to differences inherent to parity. A third possibility is that there are confounding factors responsible for the effect, such as different diets being fed to different parity cows. Regardless of the etiology of the effect, it is important to appreciate that FPR differs by parity for much of the lactation, and so parity must be taken into consideration in order to accurately interpret cows' FPR data.

The FPR is significantly higher in early lactation in cows calving in fall and winter compared to cows calving in spring and summer (Figure 4, $P < 0.05$). This effect is due to increased milk fat concentration in early lactation (Figure 9). Milk protein concentration in these cows also tends to be increased at this stage of lactation (Figure 10). The effects of season on milk fat and milk protein concentrations early in lactation are consistent with those reported in the literature (10, 77, 82).

It is apparent from the reversal in relationship between warm and cold season milk composition between early lactation test days and later lactation test days, that cows

calving in cool seasons experience warm season effects later in their lactation. This may be the reason fall calving cows have decreased FPR relative to other seasons, later in lactation – in contrast to early lactation. However, this change in relationship does not affect winter FPR, which remains significantly increased compared to other seasons at test days two and four to seven ($P < 0.05$).

The magnitude of the difference in FPR among seasons of calving is small, except at the first test day, although statistically significant at test days throughout the lactation ($P < 0.05$). The regression coefficient for summer calving cows at test day one is relatively large (r = -0.075), compared to other seasons and test days (r = -0.039 to 0.035, Table 4). Therefore it is especially important to consider season of calving when interpreting herd FPR data in the summer and when the mean DIM is low, suggesting a high proportion of recently calved cows in the herd.

The mean FPR increases linearly with parity in cows calving in all seasons except summer, where second parity cows have the lowest mean FPR. This explains the significant parity x season of calving interaction ($P = 0.0264$).

The effect of peak milk production on FPR is quadratic (Figure 6, $P < 0.0001$). This is also illustrated in Figure 5, where FPR increases for most of the later test days with increasing peak milk production but the highest peak milk production category has lowest FPR. This may be because increased peak milk production is associated with high levels of concentrate or lipid feeding, which are known to influence milk composition (12, 13, 25). Lipid feeding, in particular, would explain the simultaneous depression in milk fat and protein concentrations (Figures 11 and 12) (12, 79).

The FPR and milk fat concentration are increased and milk protein concentration decreased in cows with peak milk production ≥36.3 kg relative to <36.3 kg at the first test day (Figures 5, 11, and 12). The FPR is also increased and milk protein decreased at the second test day. The FPR and milk fat concentration are known to increase and milk protein concentration decrease with negative energy balance (1, 2). Therefore FPR may differ between peak milk production categories at the first and second test days because of differing energy balances. The effect seems restricted to early lactation because cows with the highest peak milk production have the lowest FPR at test days three to ten. This seems plausible because energy requirements are known to exceed energy intake for the first eight to ten weeks of lactation (86).

For these reasons, individual cows' peak milk production must be considered when interpreting a herd's FPR. It is safe to assume that some nutritional factors are confounding the peak milk production effect seen here.

In summary, there is likely to be misinterpretation of a herd's FPR data without prior consideration of the test day number (or DIM), parity, season of calving, and peak milk production of cows. In order to use these results in the field, the authors suggest identifying FPR data in herds of interest by these variables, and comparing herd means to those of typical herds, illustrated in Figures 2 to 5.

CONCLUSIONS

Milk FPR varies significantly among herds, likely because of differences in herd nutrition. In addition, it is affected by test day number, parity, season of calving, and peak milk production. The relationship between FPR and peak milk production is quadratic. Therefore, assessment of a herd's FPR distribution must take DIM, parity, season of

calving, and peak milk production of cows into consideration, in addition to the major dietary ingredients fed in the herd that are known to affect milk composition. The proportion of total FPR variation due to herd factors is large, but small for parity, season of calving, and peak milk production effects. There are several suggestions that a negative relationship between FPR and energy balance exists in early lactation.

CHAPTER TWO

THE USE OF REGRESSION COEFFICIENTS TO COMPARE MILK FAT-PROTEIN CONCENTRATION RATIOS AMONG DAIRY HERDS

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ABSTRACT

This report describes how regression coefficients, calculated from a statistical model including known significant variability factors (test day number, parity, season of calving, and peak milk production) affecting the ratio of milk fat concentration to protein concentration (FPR) of dairy cows, are applied to actual FPR data, within common spreadsheet software. By doing so, this application adjusts actual FPR for the effects of these variability factors. DHIA test day mean of adjusted FPR can be used to assess risk of negative energy balance, metabolic disease, and infertility in a herd. Differences in adjusted mean FPR among client's herds can be identified with the application and likely exist because of differences in nutrition, particularly nutritional factors affecting milk composition. Hence the application, which is available from the first author, will be useful in problem solving and monitoring herd nutrition.

INTRODUCTION

The FPR has been reported to be associated with dairy cows' nutritional status. Strong negative correlations between energy balance and FPR (1, 2) suggest that FPR may be useful to monitor nutritional status of cows in early lactation – a time when negative energy balance is common (86).

Associations between FPR and displaced abomasa (5, 6) and FPR and ketosis (4) suggest that FPR may also be useful as a predictor of the risk of these diseases in a herd. Additionally, there is a negative relationship between FPR and fertility (4, 7, 8).

To illustrate the use of FPR for diagnosis of displaced abomasa, cows' with first test day FPR $\geq$ 1.39 were 8.2 more likely to be subsequently diagnosed with displaced abomasa than cows' with FPR < 1.39 (95% confidence interval 2.7 to 25.0, $P < 0.05$) (5). This study design accounted for herd, parity, season, and DIM variation in FPR. Test day refers to the day on which DHIA takes milk samples from cows and these are numbered in the order in which they occur after calving, so are proportional to DIM. Sensitivity of a FPR value of $\geq$1.39 in diagnosis of subsequent displaced abomasum was 80% and specificity was 68% (5). Also, the odds ratio for subsequent ketosis, after first test day FPR > 1.5, was 3.2 ($P < 0.05$), with consideration of herd and parity effects (4).

In summary, individual herd's FPR may be a useful index by which to measure energy balance, fertility, and the risk of metabolic disease in recently calved cows. However, the herd mean FPR cannot be compared to other herds' mean FPR without prior adjustment for factors by which it is known to vary. These factors are test day number, parity, season of calving, and peak milk production (Chapter One). The effect of peak milk production is quadratic and the effect of parity varies by season of calving

(Chapter One). The difference among herds' mean FPR after adjustment for these factors is likely because of herd determinants of milk composition, the most important of which is nutrition. Other determinants are genetics and environment.

Important nutritional factors affecting milk fat concentration are the proportion of concentrates, unsaturated lipid or total lipid in the diet (12, 13, 25). Milk protein concentration varies with level of energy intake (62, 63), dietary carbohydrate characteristics (13, 74), and dietary lipid content (12, 79). There has been no research directly examining the affect of these diets on FPR. However, it is safe to extrapolate from milk composition research that FPR is commonly low within a herd when the proportion of concentrates, unsaturated, or total lipid in the diet is great (because milk fat concentration is often decreased). The mechanisms by which this occurs have not been fully elucidated, but are associated with either increased dietary supply or rumen production of *trans*-isomers of oleic acid (25). The later is likely to occur with rumen acidosis (22). Predictions of how nutrition will affect FPR are not always clear, because milk fat and protein concentrations are often affected simultaneously. For example, both milk fat and protein concentrations can be decreased by dietary lipid, which if occurs to the same extent, would not change FPR.

The objective of this report is to describe the creation of a spreadsheet that will adjust individual cow FPR for known, significant variability factors, excluding nutritional and other herd factors, and calculate basic statistics of this adjusted FPR data at each of the first ten test days.

MATERIALS AND METHODS

Production data from 4359 Holstein cow lactations, in 68 Michigan herds, were used to generate a repeated measures multivariable regression model. The purpose of the model was to identify variability factors affecting FPR at the first ten test days after calving, excluding nutrition-related variables. The model is described in Chapter One.

The herds used in the model were volunteers in another study that measured the prevalence and severity of lameness in Michigan Holstein herds. The herds in that study were selected randomly from respondents (n = 388) to a descriptive letter sent to all DHIA subscribing herd owners within a 120-km radius of Lansing, Michigan (n = 612), but who were milking 50 to 300 cows.

The last lactation for cows calving between July 1, 1997 and June 30, 1998, with milk fat and protein concentration data for the first ten test days, was selected. Cows were eliminated if the DIM at any of the first three test days were extreme for that test day (i.e. less than 7 days for the first test day, less than the 2.5^{th} percentile for the second and third test days, and greater than the 90^{th} percentile for the first three test days). Cows were excluded if milk composition was estimated, rather than measured, on any test day, or if the sampling procedure was recorded as abnormal. Examples of when milk composition is estimated are when samples freeze or sour in transit. Fourteen herds were excluded because these criteria reduced the number of cows in each of them to less than 20.

Parity was classified categorically at three levels in the model (parity one, parity two, and parity three or greater). Season of calving was classified categorically at four levels; summer (June 21 to September 21), fall (September 22 to December 21), winter

(December 22 to March 19), and spring (March 20 to June 20). Peak milk production was the greatest milk weight at any of the first ten DHIA test days.

The model demonstrated that FPR varied significantly among herds ($P < 0.0001$), by test day number ($P < 0.0001$), by parity of cows ($P < 0.0001$), season of calving of cows ($P = 0.0091$), and peak milk production ($P < 0.0001$). The effect of peak milk production was quadratic. The effect of parity varied by season of calving ($P = 0.0264$). Shapiro-Wilk tests indicated that FPR was normally distributed at all test days (W = 0.83 to 0.99) (84). Residuals from the model were also normally distributed (W = 0.80 to 0.99).

A second model was created, with the intent of calculating regression coefficients for significant variability factors from the first model, excluding the herd effect. The model specification was identical to the first model, but excluded the independent variable herd.

The mathematical relationship that describes the use of these regression coefficients to adjust measured FPR for the effect of parity, season of calving, and peak milk production, is described in Equation 1. The coefficients A, B, C, D, and E refer to the regression coefficients for the parity, season of calving, peak milk production (α), the square of peak milk production, and the parity x season of calving effects, respectively, at test day number i after calving (i = one to ten). Peak milk weights are in English units (pounds) because that is what DHIA uses. Adjustment of measured FPR allows comparison of cows or groups of cows when they differ in parity, season of calving, and peak milk production.

$$\text{Adjusted FPR}_i = \text{Measured FPR}_i - A_i - B_i - (C_i \times \alpha_i) - (D_i \times (\alpha_i)^2) - E_i$$

(Equation 1)

A Microsoft® Excel[2] spreadsheet application was created to automate the calculation of adjusted FPR (Equation 1) for the most recent test day of cows in a herd of interest, and also to calculate basic descriptive statistics of these values. This application is designed to import cow data in comma-delimited, fixed length format, contained within files named Drmscows.d1 and Drmscows.d2. These files are created by PCDART[3] software when report number 112 is run within that programme, and are stored in the herd folder within the PCDART directory. However, the application is not specific to this software and can be used with any comma-delimited, fixed length data providing that the data are contained within two files, similarly named, and in the format of Table 5. Numerical values are padded to the left with zeros (e.g. 00012) and text values are padded to the right with spaces (e.g. "f_"). Each line of data is terminated by a semicolon (ASCII character 59), carriage-return (ASCII character 13), linefeed (ASCII character 10) (i.e. ;CRLF) combination. Lines are read one at a time. Each line in the file Drmscows.d1 is 1384 bytes long, plus 3 bytes for the ;CRLF combination. Therefore the total size of this file should be evenly divisible by 1387. Each line in Drmscows.d2 is 62 bytes long, plus 3 bytes for the ;CRLF combination. Therefore the total size of this file should be evenly divisible by 65.

The application categorizes parity and season of calving in a manner similar to that used in the identification of variability factors in the first model. Peak milk production is the greatest test day milk weight for first parity cows with eight to ten test

[2] © Microsoft® Corporation, One Microsoft Way, Redmond, Washington 98052-6399
[3] Dairy Records Management Systems, 313 Chapanoke Rd., Raleigh, NC 27695

days and is the greatest test day milk weight for second or greater parity cows with four to ten test days. The means of these values, for parity x season of calving groups, are used to estimate peak milk production in first parity cows with fewer than eight test days and second or greater parity cows with fewer than four test days. Data from the first ten test days from cows' previous lactations are also used, if available, to calculate mean peak milk production.

Data exclusion criteria are similar to those used in the identification of variability factors in the first model, but in addition, cows are excluded if they have zero or missing values for parity number or calving date for the lactation, or zero or missing values for DIM, milk weight, milk fat or milk protein concentrations for any test day in the lactation. Also, cows with an eleventh or greater test day are excluded. Cows excluded by these criteria are not used in the calculation of mean peak milk production. If no data are available to calculate the mean peak milk production for a parity x season of calving group, cows which require it in Equation 1 are also excluded by the application. Once adjusted FPR is calculated for all eligible cows, the frequency, mean, and standard deviation of the mean of these values, at test days one to ten, are calculated.

RESULTS

The welcome worksheet for the application is illustrated in Figure 13. When the user clicks on the welcome panel in this worksheet, a window will open, which requests the directory path of the Drmscows.d1 and Drmscows.d2 files. The application then proceeds to calculate adjusted FPR for the most recent test day of appropriate cows. Results are listed in a worksheet that is illustrated in Figure 14. Individual cows' adjusted FPR are listed, as well as the frequency, mean, and the standard deviation of adjusted

FPR at each test day. The mean is plotted against test day number (Figure 15). To identify individual cows' adjusted FPR values, cow numbers are placed in a worksheet separate from results, but in a similar location within that worksheet to the location in the results worksheet. Cows excluded, and reason for exclusion, are also listed in this worksheet (Figure 16).

The Microsoft® Visual Basic[4] programme, used to create the Microsoft® Excel spreadsheet application, is described in appendix three. An electronic copy of the spreadsheet is available from the first author. The regression coefficients used within the application (parity, season of calving, peak milk production, the square of peak milk production, and the parity x season of calving interactions) are listed in Table 6.

DISCUSSION

This Microsoft® Excel spreadsheet application is a simple and accurate tool to adjust a herd's FPR for known, significant variability factors, excluding nutritional and other herd factors.

The herds used to generate the regression coefficients were part of a study measuring the prevalence and severity of lameness in Michigan. The high response rate (63%) to the letter requesting expression of interest in that study and random selection of herds from respondents suggest that the effect of bias is likely to be minimal and that the herds selected for the study are representative of the Mid-western United States. However, bias may be present, if, for example, herd owners experiencing lameness problems were more willing to participate than those who were not. This is important because of the associations between nutrition, milk composition, and hoof health.

[4] © Microsoft® Corporation, One Microsoft Way, Redmond, Washington 98052-6399

The data used to generate the regression coefficients did not contain cows with extreme DIM at the first three test days. The aim of this was to improve calculation of the variability factors affecting FPR at a time when milk composition is relatively dynamic, by excluding cows first enrolled in DHIA past the typical 30 DIM, and excluding cows with unusually long inter-test day time intervals early in lactation.

Approximately 80% of first parity cows in the data used to identify the variability factors affecting FPR had peak milk production prior to the ninth test day. A similar proportion of second or greater parity cows had peak milk production prior to the fifth test day. So, the application used actual peak milk production in lactations with eight to ten test days for first parity cows and four to ten test days, for second or greater parity cows. In shorter lactations, the application estimates peak milk production with the herd mean value, for the appropriate parity x season of calving group. This prevents under-estimation of peak test day milk production, in short lactations.

The regression coefficients in this application are calculated from a model containing known significant variability factors affecting FPR, excluding herd. Herd was excluded because regression coefficients for other variables are likely to less indicative of the true effect of those variables when herd is included in the model. This is because of the confounding effect of herd on these other effects. Additionally, there would be no use for herd regression coefficients in a model used diagnostically at a herd level, unless the herd of interest was one of the 68 herds used to generate the regression coefficients.

The spreadsheet excludes cows with test day eleven or greater data. This is to ensure test day ten data, from the most recent DHIA test day, reflect cows currently at

that stage of lactation, and also, because there are only regression coefficients for the first ten test days.

Calculation of mean FPR, adjusted for variability factors within a herd, is important, because FPR may be useful in the diagnosis of negative energy balance, metabolic disease, and infertility. For example, according to Geishauser et al. (1998), a value of FPR $\geq$ 1.39 at test day one, adjusted for herd, season, parity, and DIM differences, indicates 8.2 times the odds of subsequent displaced abomasa ($P < 0.05$) (5).

More importantly, this application will facilitate fair comparisons among clients' herds, by adjusting for herd differences in DIM, parity, season of calving, and peak milk production of cows. Remaining herd differences will largely reflect nutritional factors that affect milk composition, so the application will be useful in herd nutrition trouble-shooting and also as a tool to monitor changes made to herd nutritional programmes. In large herds, groups of cows could be compared among each other and also within each other, over time. Sub-normal mean FPR warrants investigation of the proportions of concentrates and lipid in the diet, and may precipitate further diagnostic procedures to confirm that a nutritional problem exists, such as ration analysis or rumenocentesis (87). This type of diagnostic investigation, combined with intensive nutritional experimentation examining dietary effects on FPR, will confirm nutritional risk factors for different levels of FPR among a herd of cows. Caution is required with interpretation of results from this application in small herds, where the frequency of test day data can be low.

CONCLUSIONS

This Microsoft® Excel spreadsheet application will allow interpretation of FPR to assess risk of negative energy balance, metabolic disease, and infertility in North American dairy herds. It will also allow fair comparisons among herds' mean FPR. The difference among herds, after their milk composition data have been processed by this application, is likely to reflect differences in herd nutrition. For this reason, it is envisaged that this application will be useful in dairy nutrition problem solving and monitoring.

APPENDICES

APPENDIX ONE

Tables

Table 1: Reports of Effects of Propionic Acid on Dry Matter Intake, Energy Intake, Milk Protein Concentration, Milk Protein Yield, Milk Yield and Serum Insulin Concentration.

Source	Control group	Treatment group	DMI[1]	MJ[2]	MP%[3]	MPY[4]	MY[5]	INS[6]
Rook and Balch, 1961(66)	water	RPA[7]	NA[8]	NA	$P < 0.001$[9]	NA	$P > 0.05$	NA
Rook et al., 1965 (67)								
Exp. I	water	RPA	NA	NA	$P < 0.05$	NA	$P > 0.05$	NA
Exp. II	water	RPA	NA	NA	$P > 0.05$	NA	$P > 0.05$	NA
Exp. III	water	RPA	NA	NA	$P < 0.05$	NA	$P > 0.05$	NA
Hurtaud et al., 1993 (52)	VFA[10] mix	RPA	$P = 0.1$	$P = 0.112$	$P = 0.594$	$P = 0.173$	$P = 0.119$	NA
Casse et al., 1994 (71)	Saline	MVP[11]	$P > 0.1$	$P > 0.1$	$P > 0.1$	NA	$P > 0.1$	$P > 0.1$
Miettinen and Huhtanen, 1996 (68)	RBA[12]	RPA	$P > 0.1$	$P > 0.1$	$P > 0.1$	$P < 0.05$	$P < 0.1$	$P > 0.1$
Huhtanen et al., 1998 (70)	RBA	RPA	$P > 0.1$	NA	$P > 0.1$	$P < 0.05$	$P < 0.05$	$P > 0.1$

[1]Dry Matter Intake, [2]Energy Intake, [3]Milk Protein Concentration, [4]Milk Protein Yield, [5]Milk yield, [6] Serum Insulin Concentration, [7]Ruminal Propionic Acid, [8]Either Not Measured or Not Statistically Analyzed, [9]Probability of Type I Error for Difference Between Treatment and Control Groups, [10]Volatile Fatty Acid, [11]Mesenteric v. Na Propionate, [12]Ruminal Butyric Acid

Table 2: Distribution of Milk Fat-Protein Concentration Ratio (FPR) and Median Days in Milk (DIM) at Test Day Numbers One to Ten After Calving (n = 4359).

Test Day Number	DIM	FPR				
	Median	Mean	SD[1]	25th Percentile	Median	75th Percentile
1	20	1.37	0.33	1.15	1.32	1.54
2	51	1.29	0.32	1.08	1.25	1.46
3	82	1.23	0.31	1.03	1.21	1.39
4	113	1.20	0.29	1.03	1.19	1.37
5	144	1.18	0.28	1.00	1.17	1.33
6	175	1.17	0.27	1.00	1.15	1.31
7	205	1.16	0.26	1.00	1.14	1.29
8	237	1.15	0.24	1.00	1.14	1.27
9	268	1.14	0.23	1.00	1.13	1.26
10	299	1.13	0.22	1.00	1.13	1.25

[1]Standard deviation

Table 3: Probabilities of Type I Error in Univariate Regression Models Examining the Variability Factors of Milk Fat-Protein Concentration Ratio at Test Day Numbers One to Ten (n = 4359).

	Variable					
Test Day Number	Herd	Parity	Season of Calving	Peak[1]	Peak x Peak	Season of Calving x Parity
1	***	***	***	***	***	NS
2	***	***	*	***	***	NS
3	***	**	NS	***	***	NS
4	***	*	*	***	***	NS
5	***	***	NS	NS	NS	NS
6	***	***	**	NS	*	**
7	***	NS	*	†	†	*
8	***	NS	NS	†	*	**
9	***	NS	NS	NS	NS	NS
10	***	*	NS	NS	NS	NS

[1]Peak Milk Production

[NS]Not Significant

†$P < 0.10$

*$P < 0.05$

**$P < 0.01$

***$P < 0.001$

Table 4: Intercept and Regression Coefficients from Repeated Measures Multivariate Regression Model Examining Variability Factors of Milk Fat-Protein Concentration Ratio, by Test Day Number (n = 4359).

	Test Day				
	1	2	3	4	5
Intercept	0.8596809065	0.7938640451	0.6995288909	0.7120921817	1.0439966980
Season of Calving					
Winter	0.0265136808	0.0227878552	0.0339712393	0.0303643293	0.0321125270
Spring	-0.0194132825	0.0213991791	0.0217563618	0.0240242792	0.0084566770
Summer	-0.0750768344	-0.0153040535	0.0292771498	0.0128197925	0.0123386320
Fall	0.0000000000	0.0000000000	0.0000000000	0.0000000000	0.0000000000
Parity					
1	-0.0538747365	-0.0824911001	-0.0302384264	-0.0249492843	-0.0664623690
2	-0.0261185027	-0.0226220342	0.0066944186	-0.0294715363	-0.0045590980
≥3	0.0000000000	0.0000000000	0.0000000000	0.0000000000	0.0000000000
Peak Milk	0.0191235786	0.0167717801	0.0147645961	0.0124237970	0.0003285891
$(\text{Peak Milk})^2$	-0.0001924084	-0.0001809762	-0.0001601731	-0.0001307544	-0.0000144251
Season of Calving x Parity					
Winter x 1	-0.0463799202	-0.0086428963	-0.0479618971	-0.0068028789	0.0058263730
Winter x 2	-0.0171093956	-0.0096126525	-0.0498971380	0.0105425250	-0.0221030480
Winter x ≥3	0.0000000000	0.0000000000	0.0000000000	0.0000000000	0.0000000000
Spring x 1	-0.0496629480	-0.0084626414	0.0049966712	-0.0007230620	0.0179873180
Spring x 2	-0.0383367321	-0.0635967028	-0.0211220038	-0.0135056560	-0.0221569960
Spring x ≥3	0.0000000000	0.0000000000	0.0000000000	0.0000000000	0.0000000000
Summer x 1	0.0031145099	0.0085447228	0.0113891349	0.0192070543	0.0154456530
Summer x 2	-0.0070136007	-0.0227992525	-0.0429072413	0.0154627296	-0.0234133040
Summer x ≥3	0.0000000000	0.0000000000	0.0000000000	0.0000000000	0.0000000000
Fall x 1	0.0000000000	0.0000000000	0.0000000000	0.0000000000	0.0000000000
Fall x 2	0.0000000000	0.0000000000	0.0000000000	0.0000000000	0.0000000000
Fall x ≥3	0.0000000000	0.0000000000	0.0000000000	0.0000000000	0.0000000000

Table 4 (cont'd).

	Test Day				
	6	7	8	9	10
Intercept	0.9830692358	0.8903831406	0.9206045921	1.1605584470	1.1239910840
Season of Calving					
Winter	0.0314474526	0.0352363357	0.0030225349	-0.0109407110	-0.0158005110
Spring	0.0180722845	-0.0080156340	-0.0114348601	-0.0219149520	0.0085162870
Summer	-0.0390814046	0.0004487161	-0.0329878540	-0.0107706970	-0.0141550780
Fall	0.0000000000	0.0000000000	0.0000000000	0.0000000000	0.0000000000
Parity					
1	-0.0763431379	-0.0209676263	-0.0181134955	-0.0226984680	0.0010154780
2	0.0007781073	-0.0001445436	-0.0130485166	-0.0296305440	-0.0081410470
≥3	0.0000000000	0.0000000000	0.0000000000	0.0000000000	0.0000000000
Peak Milk	0.0045844338	0.0051815700	0.0049659662	-0.0042502468	-0.0024134193
(Peak Milk)^2	-0.0000681754	-0.0000603617	-0.0000599107	0.0000364661	0.0000128601
Season of Calving x Parity					
Winter x 1	-0.0098924177	-0.0181164702	-0.0316530771	0.0168768160	0.0101786450
Winter x 2	-0.0256772033	-0.0080082941	-0.0073965658	0.0508224240	0.0156802210
Winter x ≥3	0.0000000000	0.0000000000	0.0000000000	0.0000000000	0.0000000000
Spring x 1	0.0140592633	0.0591740553	0.0414072299	0.0406159650	0.0263023760
Spring x 2	-0.0418132948	0.0088928832	-0.0130069792	0.0263066840	-0.0125149710
Spring x ≥3	0.0000000000	0.0000000000	0.0000000000	0.0000000000	0.0000000000
Summer x 1	0.0734455607	0.0225141582	0.0355195160	0.0053142160	0.0280716160
Summer x 2	-0.0120691438	-0.0177243296	0.0121083893	0.0180527890	0.0047448130
Summer x ≥3	0.0000000000	0.0000000000	0.0000000000	0.0000000000	0.0000000000
Fall x 1	0.0000000000	0.0000000000	0.0000000000	0.0000000000	0.0000000000
Fall x 2	0.0000000000	0.0000000000	0.0000000000	0.0000000000	0.0000000000
Fall x ≥3	0.0000000000	0.0000000000	0.0000000000	0.0000000000	0.0000000000

Table 5: Data Format Required by the Microsoft® Excel[1] Spreadsheet for Automatic Calculation of Adjusted Milk Fat-Protein Concentration Ratios.

Filename	File Item	Byte Range	Description
Drmscows.d1[2]			
	1	1-5	Cow Number
	188	1064-1065	Lactation Number
Drmscows.d2[3]			
	1	1-5	Cow Number
	2	7-14	Last Calving Date (yyyymmdd format)
	3	16-19	Test Day DIM[4]
	4	21-25	Test Day Milk (lbs)
	5	27-29	Test Day Fat %
	6	31-33	Test Day Protein %
	8	39-40	Test Day CAR Code[5]

[1]© Microsoft® Corporation, One Microsoft Way, Redmond, Washington 98052-6399

[2]Data Sorted by Increasing Cow Number

[3]Data Sorted by Increasing Cow Number, Lactation Number, then Test Day Number

[4]Days in Milk

[5]Indicates Milk Composition was Estimated or Sampling Procedure Abnormal if CAR Code = A, AF, E, F, HF, I or L

Table 6: Coefficients from a Multivariate Regression Model Created to Calculate the Effects of Within-Herd Variability Factors of Milk Fat-Protein Concentration Ratio (n = 4359).

	Test Day				
	1	2	3	4	5
Season of Calving					
Winter	0.0380050690	0.0448396405	0.0554527853	0.0570603920	0.0535406393
Spring	-0.0188552760	0.0263993901	0.0372603647	0.0370431384	0.0146490041
Summer	-0.0656567200	-0.0040356078	0.0425232782	0.0280103831	0.0292230734
Fall	0.0000000000	0.0000000000	0.0000000000	0.0000000000	0.0000000000
Parity					
1	-0.0676047670	-0.0881976182	-0.0373366317	-0.0338329669	-0.0516243493
2	-0.0237380970	-0.0166784131	0.0136658202	-0.0219904365	0.0045161050
≥3	0.0000000000	0.0000000000	0.0000000000	0.0000000000	0.0000000000
Peak Milk	0.0087975270	0.0096603770	0.0088041567	0.0077952487	0.0045685464
$(\text{Peak Milk})^2$	-0.0000441020	-0.0000524872	-0.0000482966	-0.0000425337	-0.0000258029
Season of Calving x Parity					
Winter x 1	-0.0465783670	-0.0309940456	-0.0602450814	-0.0260155412	-0.0146782289
Winter x 2	-0.0242916740	-0.0324219219	-0.0667430970	-0.0079497532	-0.0373073546
Winter x ≥3	0.0000000000	0.0000000000	0.0000000000	0.0000000000	0.0000000000
Spring x 1	-0.0749538040	-0.0535519760	-0.0346178959	-0.0348505602	-0.0119207447
Spring x 2	-0.0434922450	-0.0657729270	-0.0261952906	-0.0147891944	-0.0238383796
Spring x ≥3	0.0000000000	0.0000000000	0.0000000000	0.0000000000	0.0000000000
Summer x 1	0.0135005070	0.0099457213	0.0083336486	0.0152052947	0.0092557079
Summer x 2	-0.0147719160	-0.0329257144	-0.0545935529	-0.0025259801	-0.0413384708
Summer x ≥3	0.0000000000	0.0000000000	0.0000000000	0.0000000000	0.0000000000
Fall x 1	0.0000000000	0.0000000000	0.0000000000	0.0000000000	0.0000000000
Fall x 2	0.0000000000	0.0000000000	0.0000000000	0.0000000000	0.0000000000
Fall x ≥3	0.0000000000	0.0000000000	0.0000000000	0.0000000000	0.0000000000

Table 6 (cont'd).

	Test Day				
	6	7	8	9	10
Season of Calving					
Winter	0.0503203607	0.0540662816	0.0169699634	-0.001559981	-0.005338553
Spring	0.0291480016	-0.0011441727	-0.0006009441	-0.015123572	0.020093019
Summer	-0.0268443182	0.0150039914	-0.0265831952	-0.004079117	-0.009973458
Fall	0.0000000000	0.0000000000	0.0000000000	0.000000000	0.000000000
Parity					
1	-0.0614747988	-0.0125392022	-0.0124388433	-0.021750938	0.000165902
2	0.0137763082	0.0070289169	-0.0052774919	-0.024177437	-0.003415525
≥3	0.0000000000	0.0000000000	0.0000000000	0.000000000	0.000000000
Peak Milk	0.0059795106	0.0062768093	0.0054834073	0.002025981	0.001568476
$(\text{Peak Milk})^2$	-0.0000341495	-0.0000340785	-0.0000294815	-0.000013448	-0.000013248
Season of Calving x Parity					
Winter x 1	-0.0256687836	-0.0374551556	-0.0357256204	0.013456047	0.001009873
Winter x 2	-0.0394280675	-0.0219367068	-0.0195412581	0.040276993	0.005966185
Winter x ≥3	0.0000000000	0.0000000000	0.0000000000	0.000000000	0.000000000
Spring x 1	-0.0151174772	0.0299116676	0.0109380396	0.022566176	0.004553985
Spring x 2	-0.0469190609	0.0050518948	-0.0191053909	0.016997108	-0.022137020
Spring x ≥3	0.0000000000	0.0000000000	0.0000000000	0.000000000	0.000000000
Summer x 1	0.0691687201	0.0134193421	0.0319487935	0.000133943	0.019246534
Summer x 2	-0.0271783947	-0.0351059455	0.0009839882	0.004630118	-0.007867097
Summer x ≥3	0.0000000000	0.0000000000	0.0000000000	0.000000000	0.000000000
Fall x 1	0.0000000000	0.0000000000	0.0000000000	0.000000000	0.000000000
Fall x 2	0.0000000000	0.0000000000	0.0000000000	0.000000000	0.000000000
Fall x ≥3	0.0000000000	0.0000000000	0.0000000000	0.000000000	0.000000000

APPENDIX TWO

Figures

Figure 1: Proportion of Test Day Milk Fat-Protein Concentration Ratios < 1.0 (Inversions) vs. Test Day Number (n = 4359).

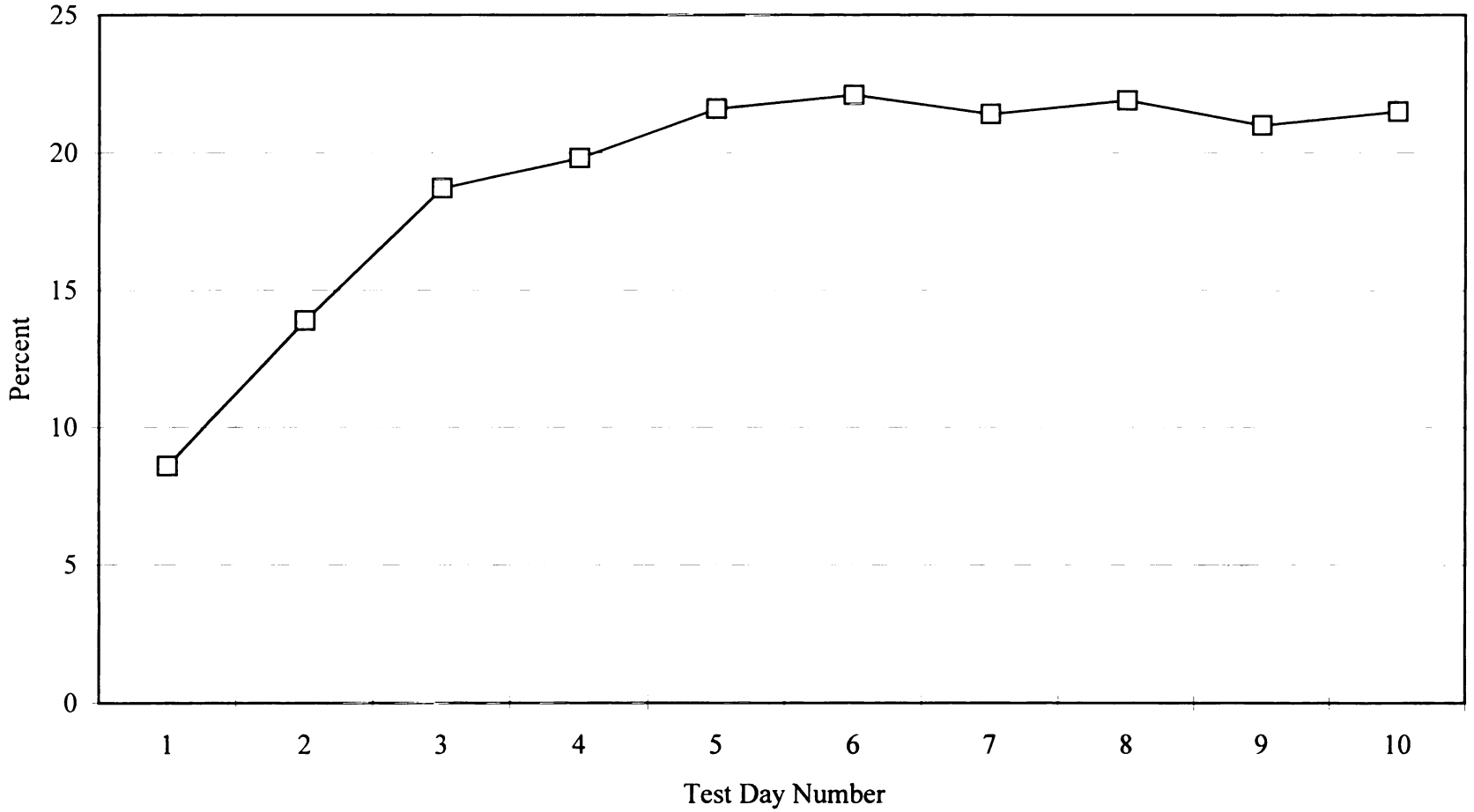

Figure 2: Herd Mean Milk Fat-Protein Concentration Ratio (FPR) (n = 68) and Mean of Herd Mean FPR (○) vs. Test Day Number.

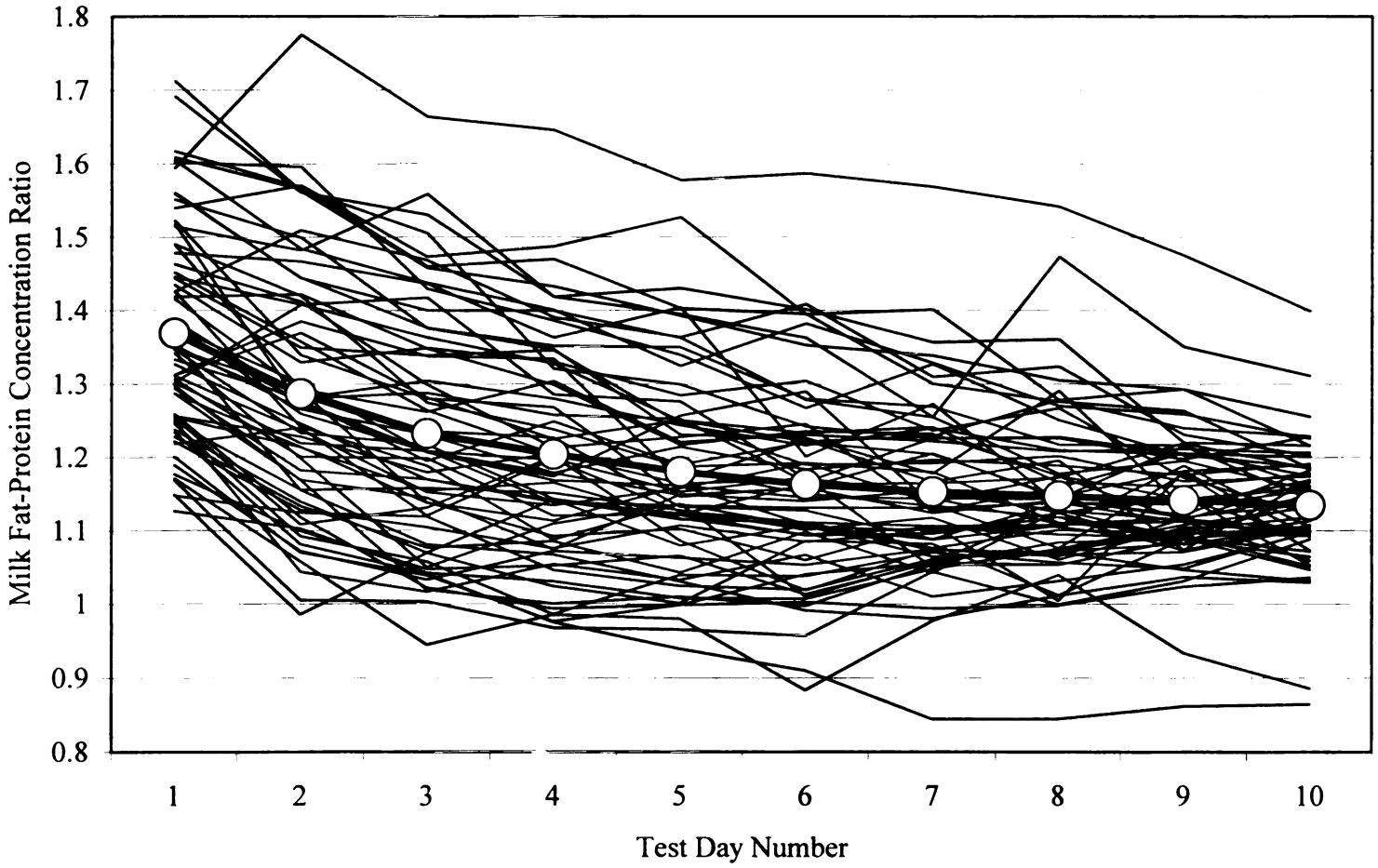

Figure 3: Milk Fat-Protein Concentration Ratio vs. Test Day Number in 68 Michigan Dairy Herds (□ = lactation 1, ○ = lactation 2, ▲ = lactation ≥3). a = lactation 1 vs. lactation 2 difference, b = lactation 1 vs. lactation ≥3 difference, c = lactation 2 vs. lactation ≥3 difference ($P < 0.05$).

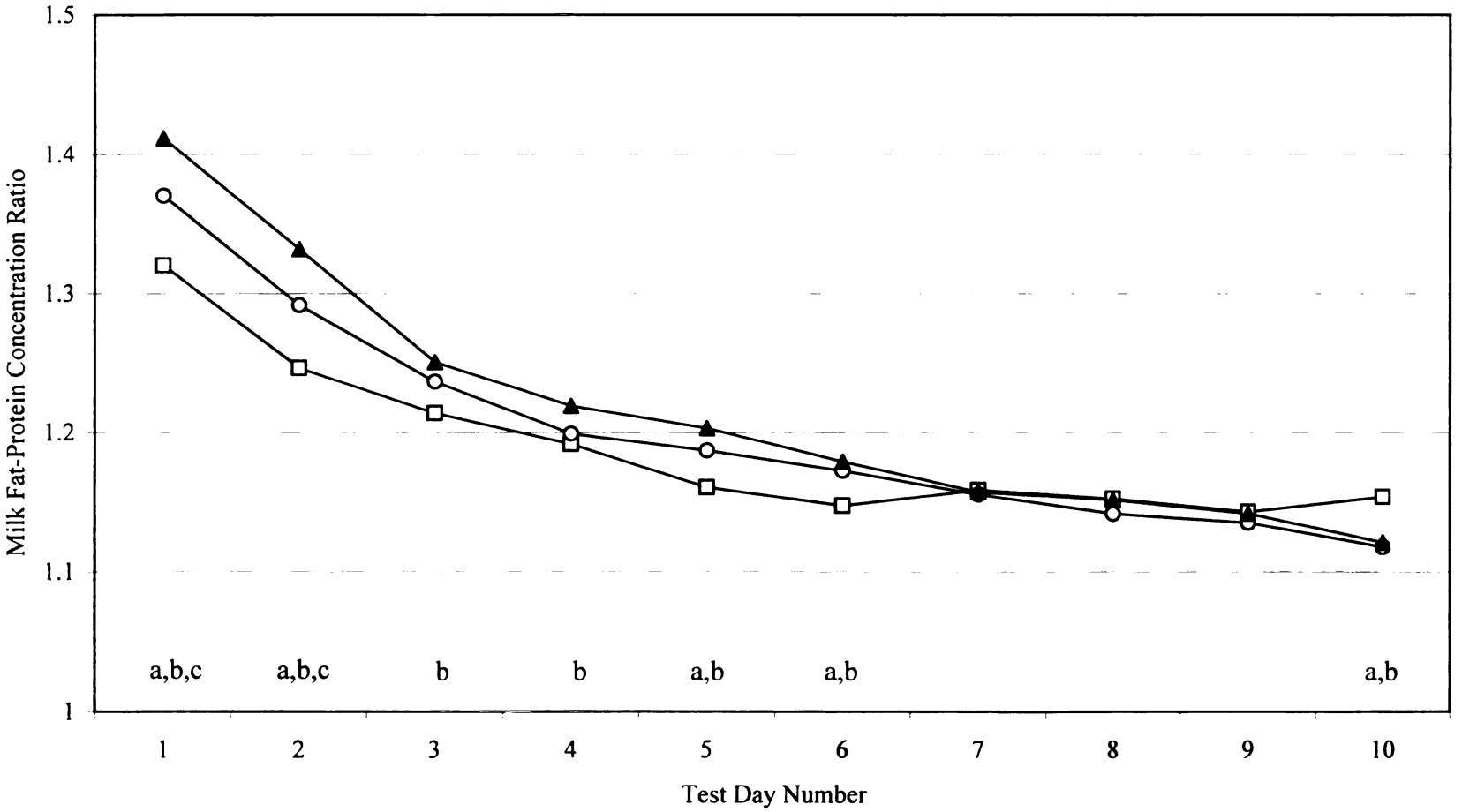

Figure 4: Milk Fat-Protein Concentration Ratio vs. Test Day Number in 68 Michigan Dairy Herds (○ = winter calving, ■ = spring calving, □ = summer calving, ● = fall calving). a = winter vs. spring difference, b = winter vs. summer difference, c = winter vs. fall difference, d = spring vs. fall difference, e = summer vs. fall difference ($P < 0.05$).

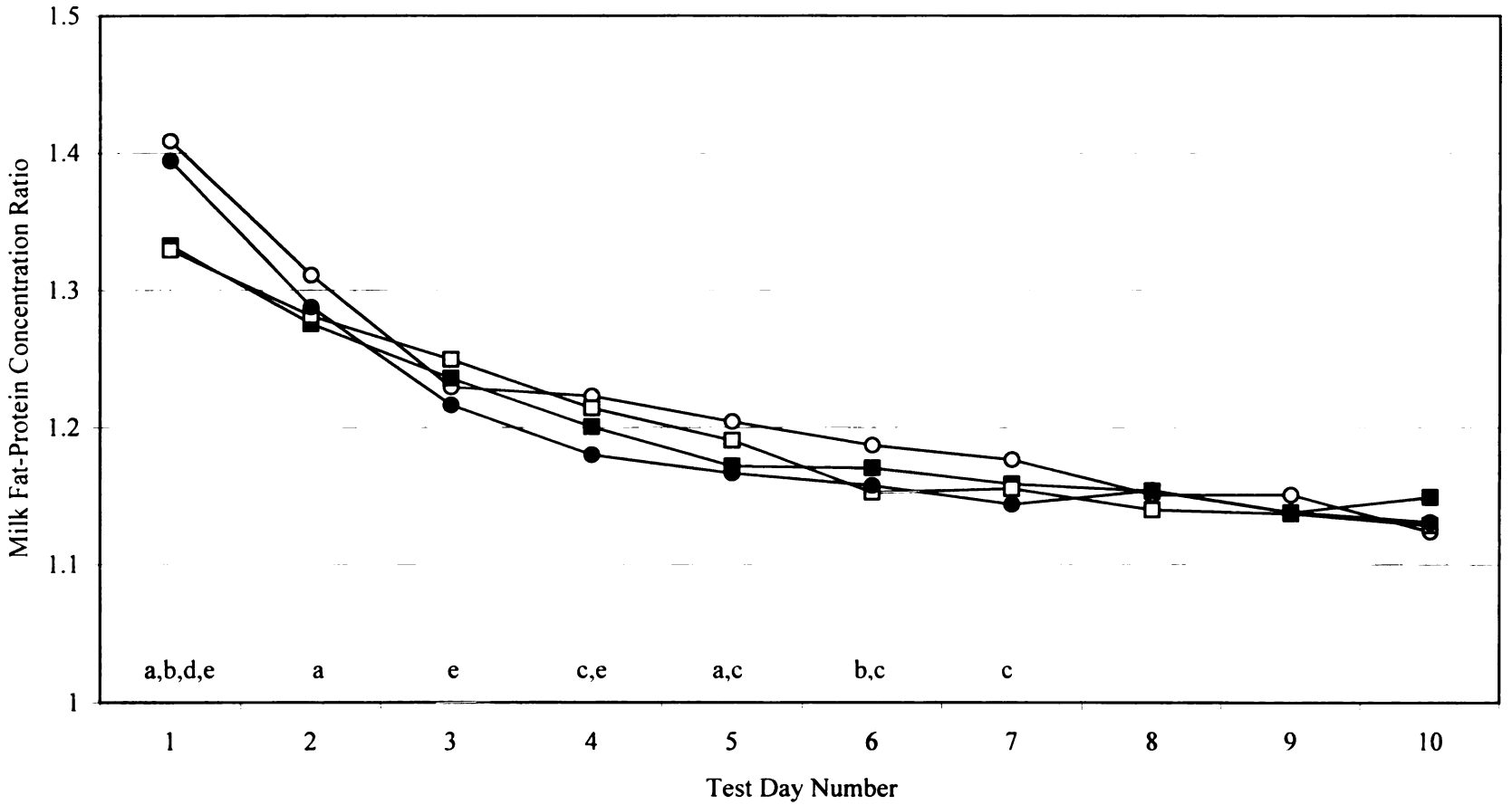

Figure 5: Milk Fat-Protein Concentration Ratio vs. Test Day Number in 68 Michigan Dairy Herds, Stratified by Peak Lactation Milk Production (○ = <36.3 kg [category 1], ■ = 36.3 kg to 45.3 kg [category 2], □ = 45.4 kg to 54.3 kg [category 3], ● = ≥54.4 kg [category 4]). a = category 1 vs. 2 difference, b = category 1 vs. 3 difference, c = category 1 vs. 4 difference, d = category 2 vs. 3 difference, e = category 2 vs. 4 difference, f = category 3 vs. 4 difference ($P < 0.05$).

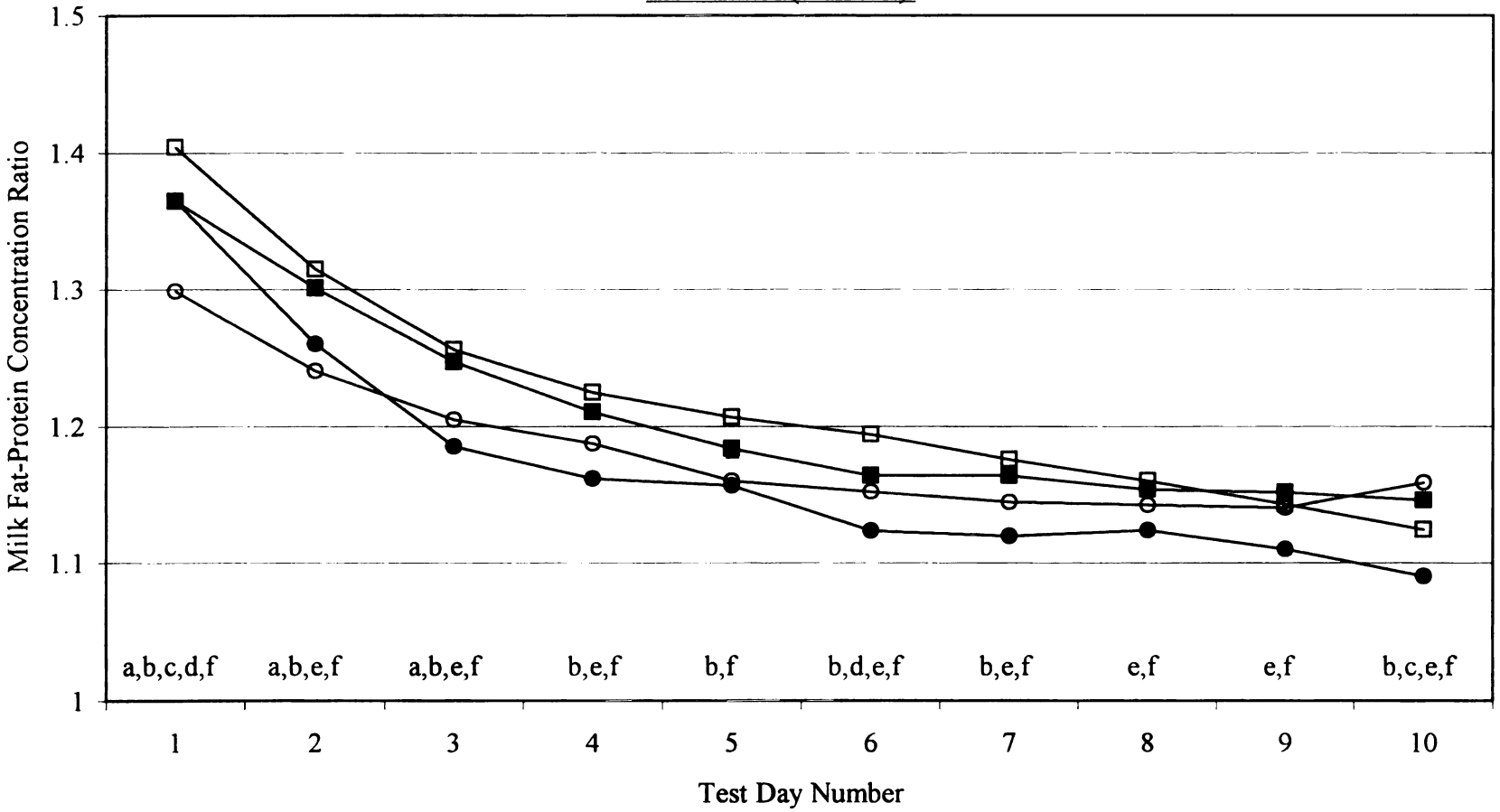

Figure 6: Milk Fat-Protein Concentration Ratio vs. Peak Lactation Milk Production (kg) (n=43,590).

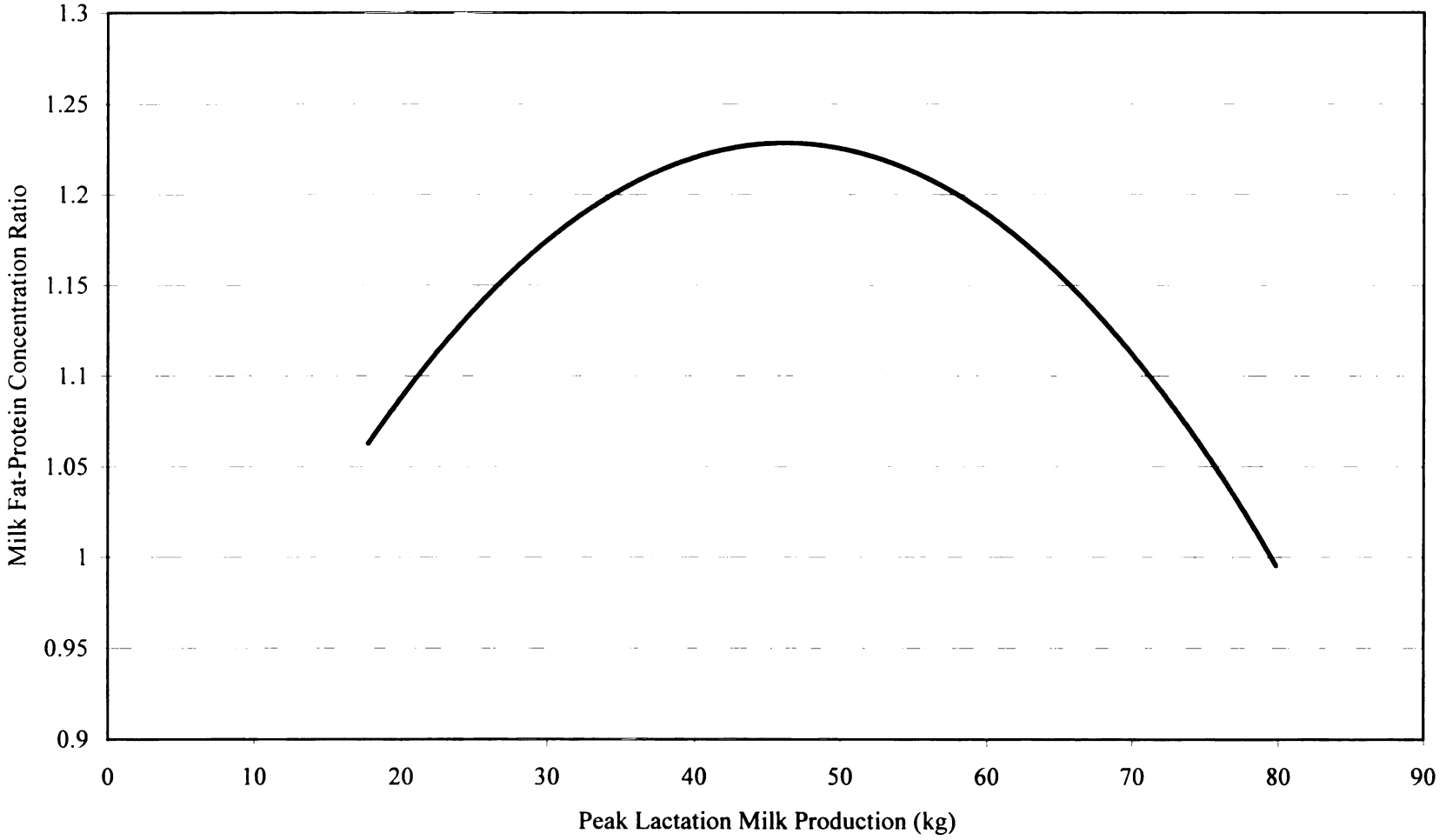

Figure 7: Milk Fat Concentration vs. Test Day Number in 68 Michigan Dairy Herds (□ = lactation 1, ○ = lactation 2, ▲ = lactation ≥3).

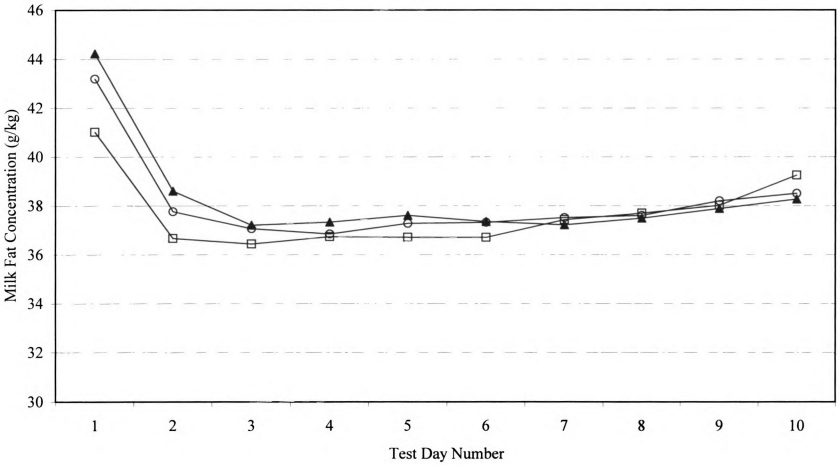

Figure 8: Milk Protein Concentration vs. Test Day Number in 68 Michigan Dairy Herds (□ = lactation 1, ○ = lactation 2, ▲ = lactation ≥3).

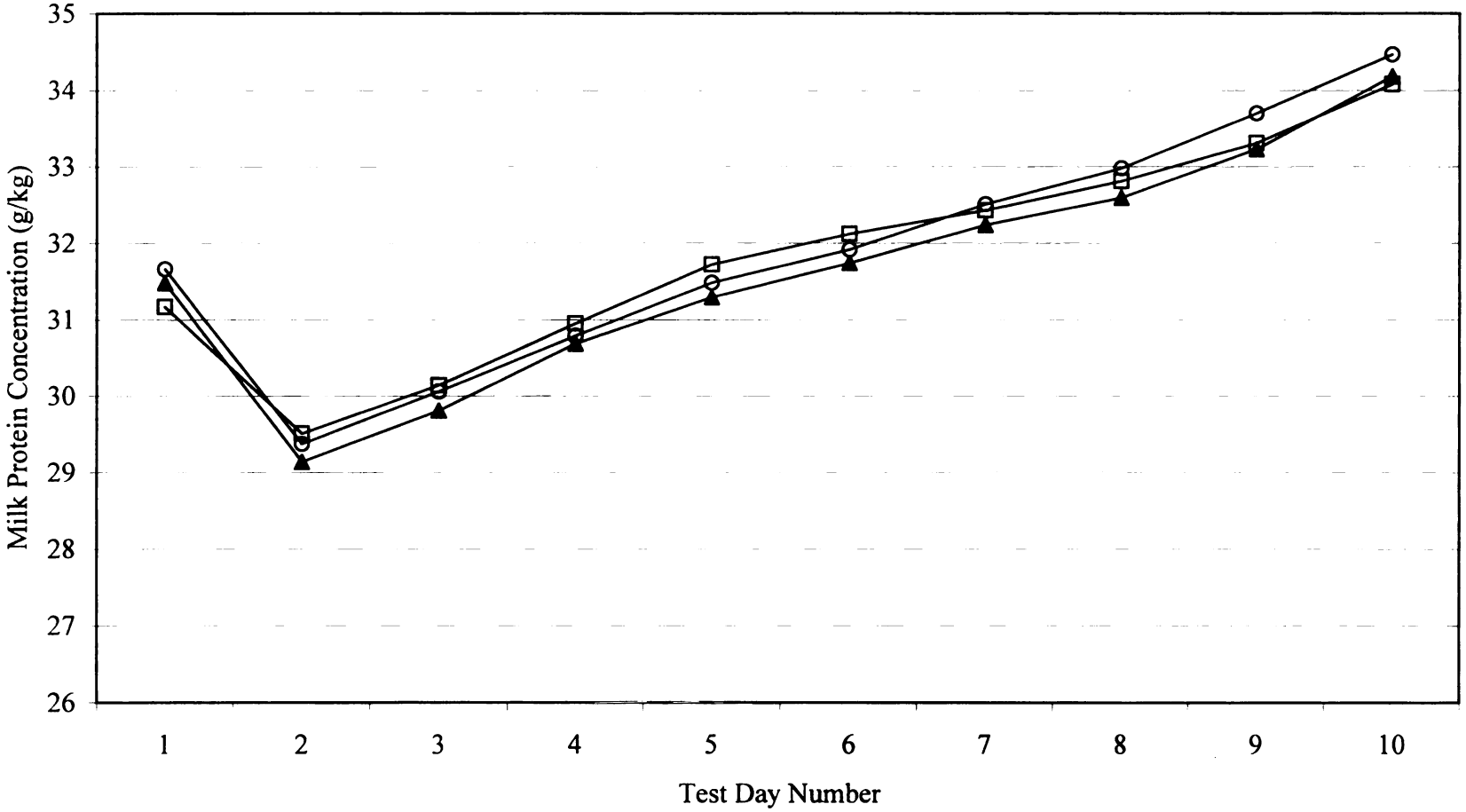

Figure 9: Milk Fat Concentration vs. Test Day Number in 68 Michigan Dairy Herds
(○ = winter calving, ■ = spring calving, □ = summer calving, ● = fall calving).

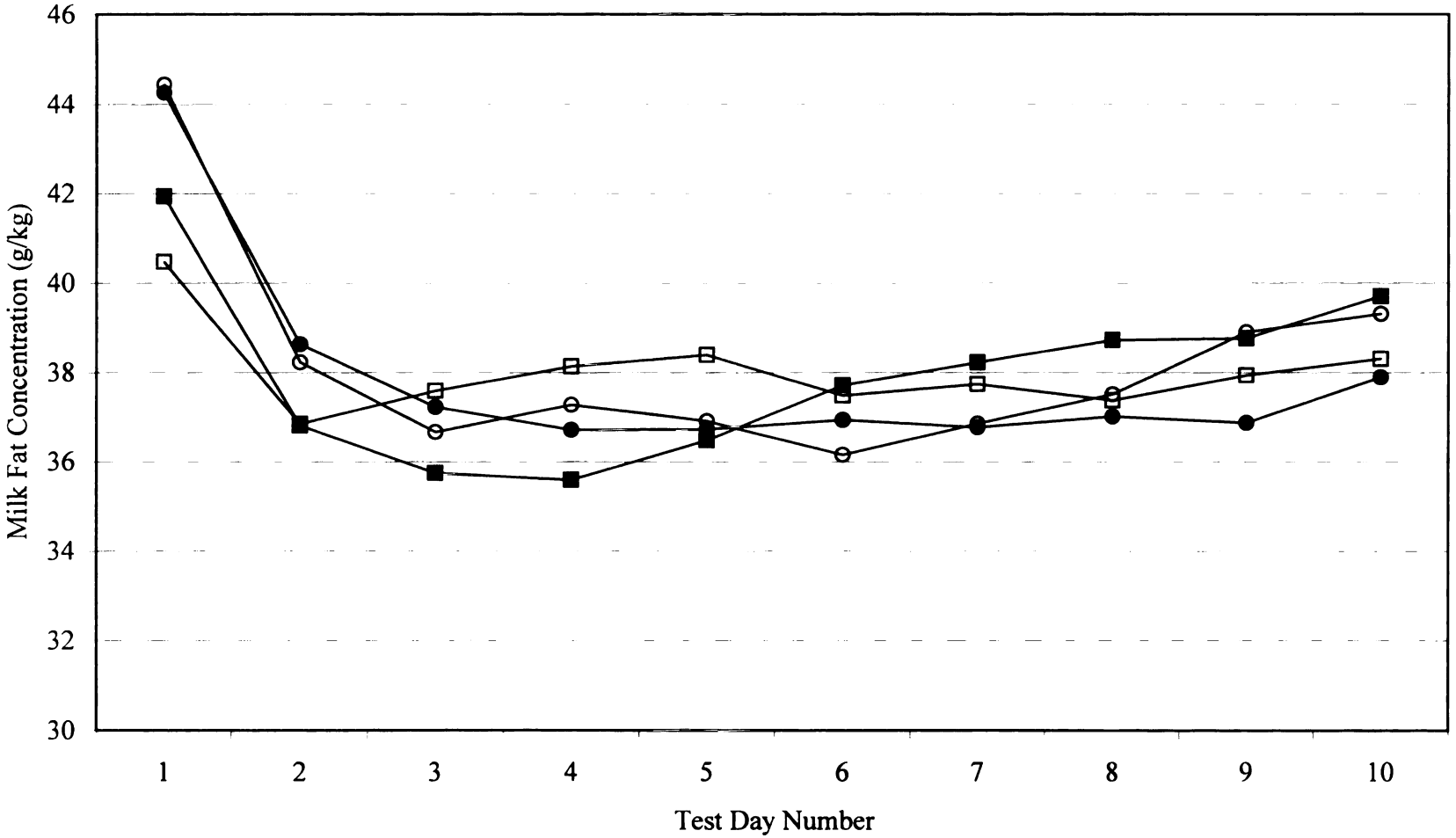

Figure 10: Milk Protein Concentration vs. Test Day Number in 68 Michigan Dairy Herds
(○ = winter calving, ■ = spring calving, □ = summer calving, ● = fall calving).

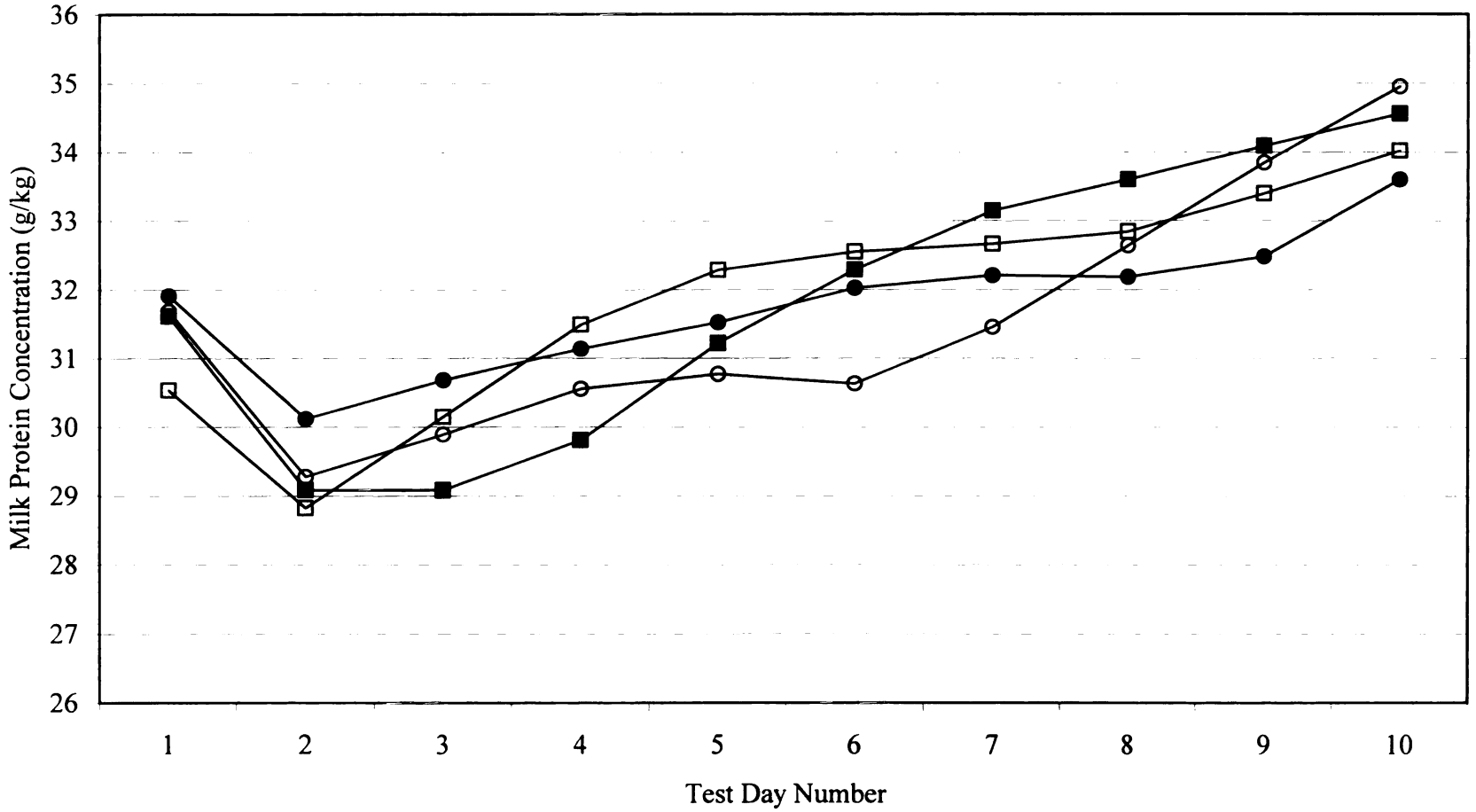

Figure 11: Milk Fat Concentration vs. Test Day Number in 68 Michigan Dairy Herds, Stratified by Peak Lactation Milk Production (○ = <36.3 kg, ■ = 36.3 kg to 45.3 kg, □ = 45.4 kg to 54.3 kg, ● = ≥54.4 kg).

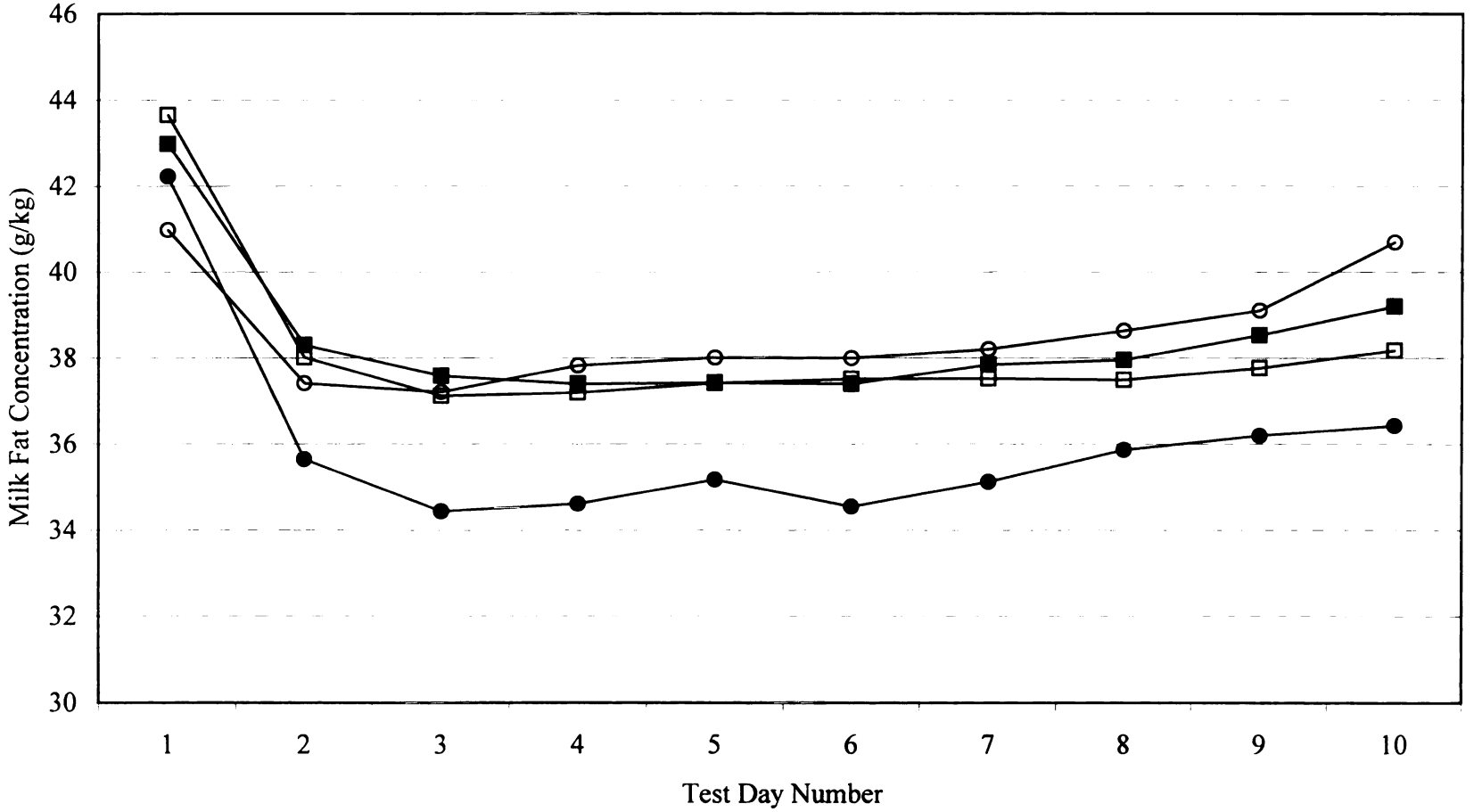

Figure 12: Milk Protein Concentration vs. Test Day Number in 68 Michigan Dairy Herds, Stratified by Peak Lactation Milk Production (○ = <36.3 kg, ■ = 36.3 kg to 45.3 kg, □ = 45.4 kg to 54.3 kg, ● = ≥54.4 kg).

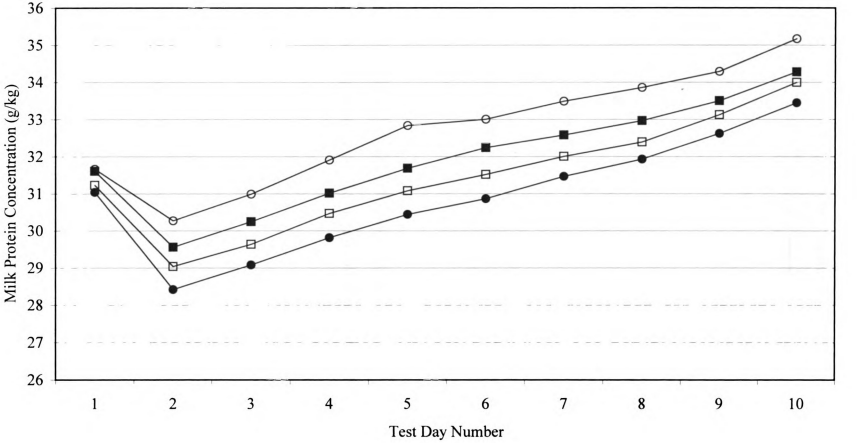

Figure 13: Welcome Worksheet in Microsoft® Excel[1] Spreadsheet Application for Automatic Calculation of Adjusted Milk Fat-Protein Concentration Ratios.

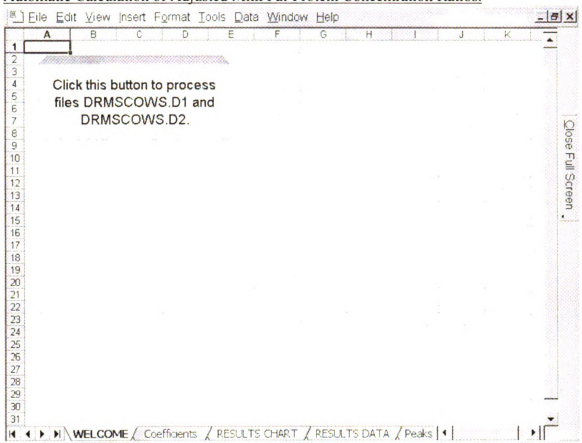

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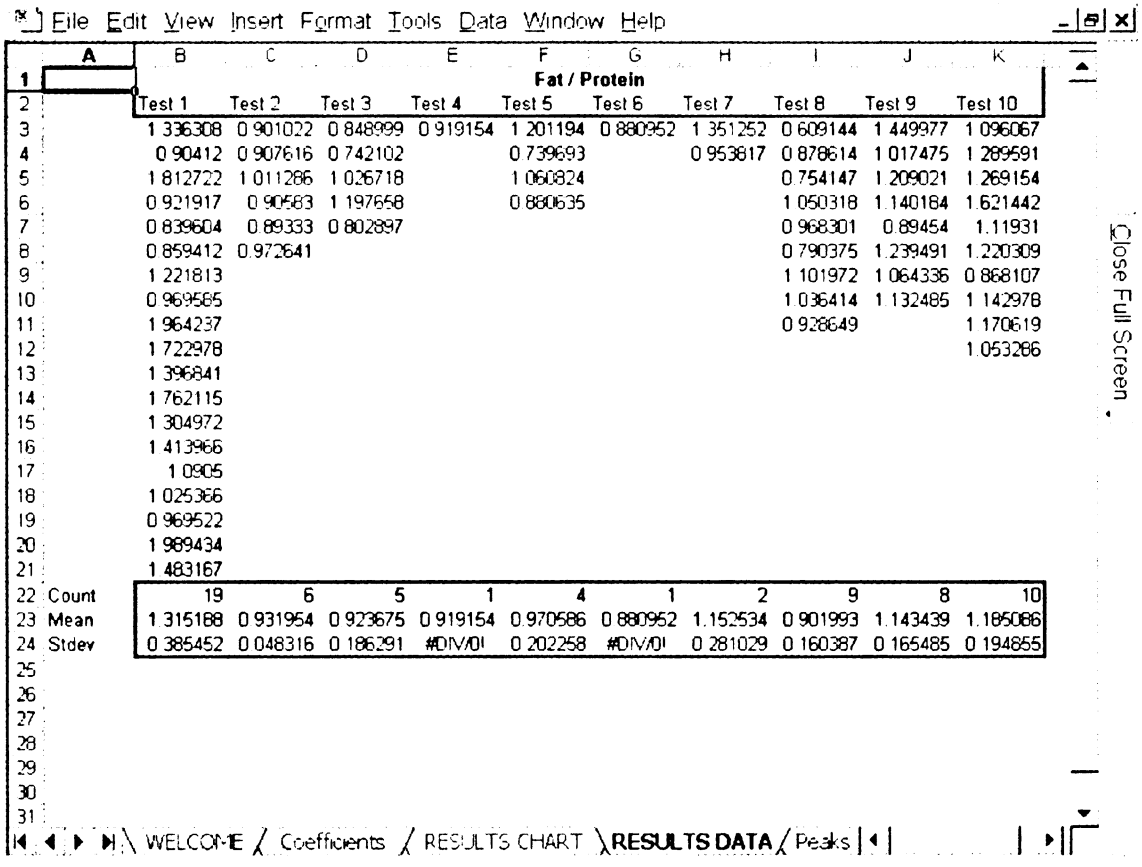
Figure 14: Results Worksheet in Microsoft® Excel[1] Spreadsheet Application for Automatic Calculation of Adjusted Milk Fat-Protein Concentration Ratios.



File Edit View Insert Format Tools Data Window Help

	A	B	C	D	E	F	G	H	I	J	K
1						Fat / Protein					
2		Test 1	Test 2	Test 3	Test 4	Test 5	Test 6	Test 7	Test 8	Test 9	Test 10
3		1 336308	0 901022	0 848999	0 919154	1 201194	0 880952	1 351252	0 609144	1 449977	1 096067
4		0 90412	0 907616	0 742102		0 739693		0 953817	0 878614	1 017475	1 289591
5		1 812722	1 011286	1 026718		1 060824			0 754147	1 209021	1 269154
6		0 921917	0 90583	1 197658		0 880635			1 050318	1 140184	1 621442
7		0 839604	0 89333	0 802897					0 968301	0 89454	1 11931
8		0 859412	0 972641						0 790375	1 239491	1 220309
9		1 221813							1 101972	1 064336	0 868107
10		0 969585							1 036414	1 132485	1 142978
11		1 964237							0 928649		1 170619
12		1 722978									1 053286
13		1 396841									
14		1 762115									
15		1 304972									
16		1 413966									
17		1 0905									
18		1 025366									
19		0 969522									
20		1 989434									
21		1 483167									
22	Count	19	6	5	1	4	1	2	9	8	10
23	Mean	1 315188	0 931954	0 923675	0 919154	0 970586	0 880952	1.152534	0 901993	1.143439	1.185086
24	Stdev	0 385452	0 048316	0 186291	#DIV/0!	0 202258	#DIV/0!	0 281029	0 160387	0 165485	0 194855
25											
26											
27											
28											
29											
30											
31											

WELCOME / Coefficients / RESULTS CHART \ **RESULTS DATA** / Peaks

Close Full Screen

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Figure 15: Results Chart in Microsoft® Excel[1] Spreadsheet Application for Automatic Calculation of Adjusted Milk Fat-Protein Concentration Ratios. This Chart Plots Herd Mean Milk Fat-Protein Concentration Ratio, Adjusted for Parity, Season of Calving, and Peak Milk Production vs. Test Day Number.

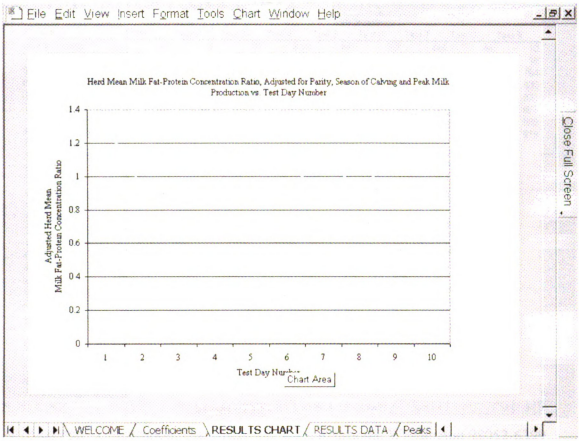

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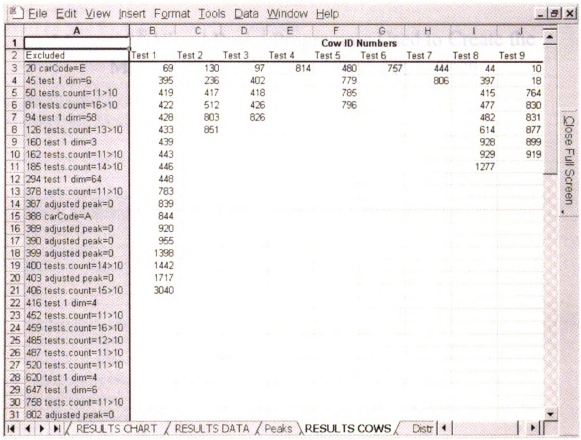
Figure 16: Cow Identity Worksheet, Describing Cows Included, Cows Excluded, and Reasons For Exclusion, in Microsoft® Excel[1] Spreadsheet Application for Automatic Calculation of Adjusted Milk Fat-Protein Concentration Ratios.



File Edit View Insert Format Tools Data Window Help

	A	B	C	D	E	F	G	H	I	J
1		Cow ID Numbers								
2	Excluded	Test 1	Test 2	Test 3	Test 4	Test 5	Test 6	Test 7	Test 8	Test 9
3	20 carCode=E	69	130	97	814	480	757	444	44	10
4	45 test 1 dim=6	395	236	402		779		806	397	18
5	50 tests.count=11>10	419	417	418		785			415	764
6	81 tests.count=16>10	422	512	426		796			477	830
7	94 test 1 dim=58	428	803	826					482	831
8	126 tests.count=13>10	433	851						614	877
9	160 test 1 dim=3	439							928	899
10	162 tests.count=11>10	443							929	919
11	185 tests.count=14>10	446							1277	
12	294 test 1 dim=64	448								
13	378 tests.count=11>10	783								
14	387 adjusted peak=0	839								
15	388 carCode=A	844								
16	389 adjusted peak=0	920								
17	390 adjusted peak=0	955								
18	399 adjusted peak=0	1398								
19	400 tests.count=14>10	1442								
20	403 adjusted peak=0	1717								
21	406 tests.count=15>10	3040								
22	416 test 1 dim=4									
23	452 tests.count=11>10									
24	459 tests.count=16>10									
25	485 tests.count=12>10									
26	487 tests.count=11>10									
27	520 tests.count=11>10									
28	620 test 1 dim=4									
29	647 test 1 dim=6									
30	758 tests.count=11>10									
31	802 adjusted peak=0									

RESULTS CHART | RESULTS DATA | Peaks | RESULTS COWS | Distr

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APPENDIX THREE

Microsoft® Visual Basic[1] Programme Used to Create the Microsoft® Excel[1] Spreadsheet Application

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```
Attribute VB_Name = "modMilk"
'milk module
'2001 by Will Raphael and Joel Dobrzelewski
'Michigan State University
'requires additional classes: clsCow, clsHerd, clsMilkTest

Option Explicit
Global Const MaximumNumberOfTests = 10

Public Sub GenerateResults()

   Dim h As clsHerd
   Dim p As String

   p = InputBox("Path", "Data Path", ActiveWorkbook.path)
   If p = "" Then Exit Sub


   Set h = New clsHerd

   With ActiveWorkbook
      h.CalculateResults p, .Sheets("Results Data"), .Sheets("Results Cows"),
.Charts("Results Chart")
      .Charts("Results Chart").Activate
   End With

   Set h = Nothing

End Sub

Public Function GetCoefficient(ByVal coefficient As Integer, ByVal row As Integer,
ByVal column As Integer) As Double

   Const leftcol = 2

   Dim toprow As Long
   Dim sheetrow As Long
   Dim sheetcol As Long

   Select Case coefficient
      Case 1
         toprow = 2
      Case 2
         toprow = 6
```

```
        Case 3
            toprow = 9
        Case 4
            toprow = 10
        Case 5
            toprow = 11
        Case Else
            'trying to retreive an invalid coefficient
            'look at the calling routine
            Stop
    End Select

    sheetrow = toprow + row - 1
    sheetcol = leftcol + column - 1

    GetCoefficient = Sheets("Coefficients").Cells(sheetrow, sheetcol)

End Function

Public Sub HighlightRegion(range1 As range, color1 As Long)

    range1.Borders(xlDiagonalDown).LineStyle = xlNone
    range1.Borders(xlDiagonalUp).LineStyle = xlNone
    With range1.Borders(xlEdgeLeft)
        .LineStyle = xlContinuous
        .Weight = xlMedium
        .colorIndex = xlAutomatic
    End With
    With range1.Borders(xlEdgeTop)
        .LineStyle = xlContinuous
        .Weight = xlMedium
        .colorIndex = xlAutomatic
    End With
    With range1.Borders(xlEdgeBottom)
        .LineStyle = xlContinuous
        .Weight = xlMedium
        .colorIndex = xlAutomatic
    End With
    With range1.Borders(xlEdgeRight)
        .LineStyle = xlContinuous
        .Weight = xlMedium
        .colorIndex = xlAutomatic
    End With
    range1.Borders(xlInsideVertical).LineStyle = xlNone
    range1.Borders(xlInsideHorizontal).LineStyle = xlNone
    With range1.Interior
```

```
    .Color = color1
    .Pattern = xlSolid
  End With

End Sub

Public Function MilkCDate(s As String) As Date

  MilkCDate = CDate(Mid(s, 5, 2) & "/" & Mid(s, 7, 2) & "/" & Mid(s, 1, 4))

End Function
```

```
VERSION 1.0 CLASS
BEGIN
  MultiUse = -1  'True
END
Attribute VB_Name = "clsCow"
Attribute VB_GlobalNameSpace = False
Attribute VB_Creatable = False
Attribute VB_PredeclaredId = False
Attribute VB_Exposed = False
'cow class
'2001 by Will Raphael and Joel Dobrzelewski
'Michigan State University

Option Explicit

Public reason As String
Public id As Long
Public lactationNumber As Long
Public lactations As Collection
Public herd As clsHerd

Public Sub addTest(test As clsMilkTest)

  Dim l As clsLactation

  'is this a new calving date?
  If test.calvingDate <> lastCalvingDate Then
    'yes this is a new calving date and a new series of tests
    Set l = New clsLactation
    l.calvingDate = test.calvingDate
    Set l.cow = Me
    lactations.Add l
  End If
```

```
    lastLactation.addTest test

End Sub

Public Function adjustedFpr() As Double

    Dim c1 As Double
    Dim c2 As Double
    Dim c3 As Double
    Dim c4 As Double
    Dim c5 As Double

    Dim fpr As Double
    Dim Peak As Double
    Dim t As Long

    t = getTestNumber
    If t = 0 Then Exit Function

    c1 = GetCoefficient(1, season, t)
    c2 = GetCoefficient(2, parity, t)
    c3 = GetCoefficient(3, 1, t)
    c4 = GetCoefficient(4, 1, t)
    c5 = GetCoefficient(5, SeasonParity, t)

    fpr = realFpr()
    Peak = adjustedPeak()

    'peak and fpr should never be zero
    'if they are, then these cows should be excluded from herd data
    'and you should not call this function
    If Peak = 0 Then Stop
    If fpr = 0 Then Stop

    adjustedFpr = (fpr - c1) - (c2) - (Peak * c3) - (Peak * Peak * c4) - c5

End Function

Private Function adjustedPeak() As Double

    Dim p As Double

    If lastLactation.goodLength Then
      adjustedPeak = lastLactation.Peak
    Else
```

```
    p = herd.Peak(parity, season)
    adjustedPeak = p
  End If

End Function

Public Function hasExcludedLactations() As Boolean

  Dim lactation As clsLactation

  For Each lactation In lactations
    If lactation.excluded Then GoTo YesExcluded
  Next

  hasExcludedLactations = False
  Exit Function

YesExcluded:

  hasExcludedLactations = True

End Function

Public Function isExcludedFromHerd()

  If lactations.count = 0 Then reason = "no lactations": GoTo YesExcluded
  If (adjustedPeak = 0) Then reason = "adjusted peak=0": GoTo YesExcluded
  If lastLactation.excluded Then reason = lastLactation.reason: GoTo YesExcluded

  isExcludedFromHerd = False
  Exit Function

YesExcluded:

  isExcludedFromHerd = True

End Function

Public Function getTestNumber() As Long

  getTestNumber = lastTest.number

End Function

Private Function lastCalvingDate() As Date
```

```
    If lactations.count > 0 Then lastCalvingDate = lastLactation.calvingDate
End Function
Public Function lastLactation() As clsLactation
    Set lastLactation = lactations(lactations.count)
End Function
Public Function lastTest() As clsMilkTest
    Set lastTest = lastLactation.lastTest
End Function
Public Function parity() As Long
    parity = lastLactation.parity
End Function
Private Function realPeak() As Double
    realPeak = lastLactation.Peak
End Function
Public Function realFpr() As Double
    realFpr = lastTest.fpr
End Function
Public Sub RemoveNullTests()
    Dim lactation As clsLactation
    Dim i As Long

    For Each lactation In lactations
        lactation.RemoveNullTest
    Next

    i = 1
    Do While i <= lactations.count
        If lactations(i).tests.count = 0 Then
```

```
        lactations.Remove i
      Else
        i = i + 1
      End If
    Loop

End Sub

Public Function season() As Long

    season = lastLactation.season

End Function

Public Function SeasonParity() As Long

    SeasonParity = (season - 1) * 3 + parity

End Function

Private Sub Class_Initialize()

    Set lactations = New Collection

End Sub

Private Sub Class_Terminate()

    Set lactations = Nothing

End Sub
```

```
VERSION 1.0 CLASS
BEGIN
  MultiUse = -1  'True
END
Attribute VB_Name = "clsHerd"
Attribute VB_GlobalNameSpace = False
Attribute VB_Creatable = False
Attribute VB_PredeclaredId = False
Attribute VB_Exposed = False
'herd class
'2001 by Will Raphael and Joel Dobrzelewski
'Michigan State University
```

```
Option Explicit

Const seasons = 4
Const parities = 3

Private cows As Collection
Private peakMilk(1 To parities, 1 To seasons) As Double
Private peaksCalculated As Boolean

Private Sub CalculatePeaks()

   Dim cow As clsCow
   Dim lactation As clsLactation
   Dim p As Long
   Dim s As Long

   Dim peaktotal(1 To parities, 1 To seasons) As Double
   Dim cowcount(1 To parities, 1 To seasons) As Double

   For Each cow In cows
      If (cow.lactations.count > 0) And (Not cow.hasExcludedLactations) Then
         p = cow.parity
         s = cow.season

         For Each lactation In cow.lactations
            If lactation.goodLength Then
               peaktotal(p, s) = peaktotal(p, s) + lactation.Peak
               cowcount(p, s) = cowcount(p, s) + 1
            End If
         Next
      End If
   Next

   For p = 1 To parities
      For s = 1 To seasons
         If cowcount(p, s) > 0 Then peakMilk(p, s) = peaktotal(p, s) / cowcount(p, s)
         Sheets("Peaks").Cells(p + 2, s + 1) = peakMilk(p, s)
         Sheets("Peaks").Cells(p + parities + 5, s + 1) = cowcount(p, s)
      Next
   Next

   peaksCalculated = True

End Sub
```

```
Public Sub CalculateResults(path As String, resultsheet As Worksheet, cowsheet As
Worksheet, resultchart As Chart)

  Const tests = 10
  Const header = 2

  Dim cow As clsCow
  Dim last(0 To tests)
  Dim t As Long
  Dim r As String
  Dim footer As Long

  'load data into herd and cow objects
  LoadHerd path

  'clear the results sheets
  footer = 0
  resultsheet.Cells.Clear
  cowsheet.Cells.Clear

  'process the herd
  For Each cow In cows

    'make sure the cow is valid
    If Not cow.isExcludedFromHerd Then

      t = cow.getTestNumber

      'keep track of the number of cows in this test
      last(t) = last(t) + 1

      'display the adjusted FPR value
      resultsheet.Cells(last(t) + header, t + 1) = cow.adjustedFpr

      'display the cow ID number
      cowsheet.Cells(last(t) + header, t + 1) = cow.id

      'keep track of where the footer will go
      If last(t) > footer Then footer = last(t)
    Else
      'this cow is rejected
      last(0) = last(0) + 1
      cowsheet.Cells(last(0) + header, 1) = cow.id & " " & cow.reason
    End If
  Next
```

```
  footer = footer + header + 1

  'display the test results

  For t = 1 To tests
    'temporary range for statistical calculations
    r = Chr(t + 65) & "2:" & Chr(t + 65) & footer - 1

    resultsheet.Cells(2, t + 1) = "Test " & t
    cowsheet.Cells(2, t + 1) = "Test " & t

    resultsheet.Cells(footer + 0, t + 1) = "=count(" & r & ")"
    resultsheet.Cells(footer + 1, t + 1) = "=average(" & r & ")"
    resultsheet.Cells(footer + 2, t + 1) = "=stdev(" & r & ")"

  Next

  'finish up the result sheet

  With resultsheet
    .Cells(1, 2) = "Fat / Protein"
    .Cells(1, 2).Font.Bold = True

    .range(.Cells(1, 2), .Cells(1, tests + 1)).Merge
    .range(.Cells(1, 2), .Cells(1, tests + 1)).HorizontalAlignment = xlCenter

    .Cells(footer + 0, 1) = "Count"
    .Cells(footer + 1, 1) = "Mean"
    .Cells(footer + 2, 1) = "Stdev"

    HighlightRegion .range(.Cells(1, 2), .Cells(header, tests + 1)), RGB(255, 255, 153)
    HighlightRegion .range(.Cells(footer, 2), .Cells(footer + 2, tests + 1)), RGB(204,
255, 204)
  End With

  'finish up the cow id sheet

  With cowsheet
    .Cells(1, 2) = "Cow ID Numbers"
    .Cells(2, 1) = "Excluded"
    .Cells(1, 2).Font.Bold = True

    .range(.Cells(1, 2), .Cells(1, tests + 1)).Merge
    .range(.Cells(1, 2), .Cells(1, tests + 1)).HorizontalAlignment = xlCenter

    HighlightRegion .range(.Cells(1, 2), .Cells(header, tests + 1)), RGB(255, 255, 153)
```

```
    HighlightRegion .range(.Cells(header + 1, 1), .Cells(last(0) + header, 1)), RGB(255,
153, 204)
  End With

  Dim oldblanks As Long

  'update the graph
  With resultchart
    'tricky problem: can't change formula if the series is blank and visible
    oldblanks = .DisplayBlanksAs
    .SeriesCollection("This Herd").FormulaR1C1 = _
      "=SERIES(""This Herd"",'Results Data'!R" & footer + 1 & "C2:R" & footer + 1
& "C" & tests + 1 & ",1)"
    'return old visibility
    .DisplayBlanksAs = oldblanks
  End With

End Sub

Private Sub LoadCows(path As String)

  Dim f As Integer
  Dim s As String
  Dim c As clsCow

  'get a free file handle
  f = FreeFile

  'open the cow file
  On Error Resume Next
  Open path & "\DRMSCOWS.D1" For Input As f
  If Err.number <> 0 Then
    Err.Clear
    Open path & "\DRMSCOWS.D1.csv" For Input As f
    If Err.number Then Exit Sub
  End If
  On Error GoTo 0

  Do Until EOF(f)
    'read a line at a time
    Line Input #f, s

    'add the cow to the herd
    Set c = New clsCow

    With c
```

```
            .id = CLng(Mid(s, 1, 5))
            .lactationNumber = CLng(Mid(s, 1064, 2))
            Set .herd = Me
        End With

        cows.Add c, "i" & c.id
    Loop

    'close the file
    Close f

End Sub

Private Sub LoadHerd(path As String)

    LoadCows path
    LoadTests path
    RemoveNullTests
    UpdateLactationNumbers
    CalculatePeaks

End Sub

Private Sub LoadTests(path As String)

    Dim c As clsCow
    Dim f As Integer
    Dim s As String
    Dim t As clsMilkTest

    'get a free file handle
    f = FreeFile

    'open the milk test file
    On Error Resume Next
    Open path & "\DRMSCOWS.D2" For Input As f
    If Err.number <> 0 Then
        Err.Clear
        Open path & "\DRMSCOWS.D2.csv" For Input As f
        If Err.number <> 0 Then Exit Sub
    End If
    On Error GoTo 0

    Do Until EOF(f)
        'read a line at a time
        Line Input #f, s
```

```
        'create a new milk test
        Set t = New clsMilkTest

        t.cow = CLng(Mid(s, 1, 5))
        t.calvingDate = MilkCDate(Mid(s, 7, 8))
        t.daysInMilk = CDbl(Mid(s, 16, 4))
        t.milk = CDbl(Mid(s, 21, 5))
        t.fat = CDbl(Mid(s, 27, 3))
        t.protein = CDbl(Mid(s, 31, 3))
        t.carCode = Trim(Mid(s, 39, 2))

        'add it to the collection of tests
        On Error Resume Next
        Set c = cows("i" & t.cow)
        If Err.number > 0 Then
           'found a test with no corresponding cow
           'this should never happen
           'all cows should be listed in drmscows.dl
           Stop
        End If
        On Error GoTo 0

        cows("i" & t.cow).addTest t
    Loop

    Close f

End Sub

Public Function Peak(parity As Long, season As Long) As Double

    'should not be trying to retreive the peak if they have not been calculated yet
    'something must have gone wrong
    If Not peaksCalculated Then Stop

    Peak = peakMilk(parity, season)

End Function

Private Sub RemoveNullTests()

    Dim cow As clsCow
    Dim i As Long

    i = 1
```

```
    Do Until i > cows.count
        cows(i).RemoveNullTests
        If cows(i).lactations.count = 0 Then
            cows.Remove i
        Else
            i = i + 1
        End If
    Loop

End Sub

Private Sub UpdateLactationNumbers()

    Dim cow As clsCow
    Dim lactation As clsLactation
    Dim i As Long

    For Each cow In cows
        i = cow.lactationNumber - cow.lactations.count + 1

        For Each lactation In cow.lactations
            lactation.number = i
            i = i + 1
        Next
    Next

End Sub

Private Sub Class_Initialize()

    Set cows = New Collection

End Sub

Private Sub Class_Terminate()

    Set cows = Nothing

End Sub
```

```
VERSION 1.0 CLASS
BEGIN
  MultiUse = -1  'True
END
```

```
Attribute VB_Name = "clsLactation"
Attribute VB_GlobalNameSpace = False
Attribute VB_Creatable = False
Attribute VB_PredeclaredId = False
Attribute VB_Exposed = False
'lactation class
'2001 by Will Raphael and Joel Dobrzelewski
'Michigan State University

Option Explicit

Public reason As String

Public calvingDate As Date
Public Peak As Double
Public tests As Collection

Public cow As clsCow
Private num As Long

Public Sub addTest(test As clsMilkTest)

   test.number = tests.count + 1
   tests.Add test
   If test.milk > Peak Then Peak = test.milk

End Sub

Public Function excluded() As Boolean

   Dim test As clsMilkTest

   If tests.count < 1 Then
      reason = "tests.count<1"
      GoTo YesExcluded
   End If

   If tests.count > MaximumNumberOfTests Then
      reason = "tests.count=" & tests.count & ">" & MaximumNumberOfTests
      GoTo YesExcluded
   End If

   For Each test In tests
      If Not test.isValid Then
         reason = test.reason
         GoTo YesExcluded
```

```
      End If
   Next

   excluded = False
   Exit Function

YesExcluded:

   excluded = True

End Function

Public Function goodLength() As Boolean

   'assume failure
   goodLength = False

   If tests.count <= MaximumNumberOfTests Then
      Select Case parity
         Case 1
            If tests.count >= 8 Then goodLength = True
         Case 2, 3
            If tests.count >= 4 Then goodLength = True
         Case Else
            'invalid parity - should not happen
            'could be a file problem
            Stop
      End Select
   End If

End Function

Public Function lastTest() As clsMilkTest

   Set lastTest = tests(tests.count)

End Function


Public Property Get number() As Long

   'all tests should be numbered
   'must be a logic problem when tests were added
   If num = 0 Then Stop

   number = num
```

```
End Property

Public Property Let number(value As Long)

   num = value

End Property

Public Function parity() As Long

   Select Case num
      Case 1
         parity = 1
      Case 2
         parity = 2
      Case Is >= 3
         parity = 3
      Case Else
         parity = 0
   End Select

End Function

Public Sub RemoveNullTest()

   If lastTest.fat = 0 And lastTest.protein = 0 And lastTest.milk = 0 Then
      tests.Remove tests.count
   End If

End Sub

Public Function season() As Long

   Const winter = 356 'dec 22
   Const spring = 79  'mar 20
   Const summer = 172 'jun 21
   Const autumn = 265 'sep 22

   Dim d As Long

   d = DatePart("y", lastTest.calvingDate)

   Select Case d
      Case winter To 366, 1 To spring - 1
         season = 1
```

```
    Case spring To summer - 1
      season = 2
    Case summer To autumn - 1
      season = 3
    Case autumn To winter - 1
      season = 4
    Case Else
      season = 0
      'invalid calving date?
      Stop
  End Select

End Function

Private Sub Class_Initialize()

  Set tests = New Collection
  Peak = 0

End Sub

Private Sub Class_Terminate()

  Set tests = Nothing

End Sub
```

```
VERSION 1.0 CLASS
BEGIN
  MultiUse = -1  'True
END
Attribute VB_Name = "clsMilkTest"
Attribute VB_GlobalNameSpace = False
Attribute VB_Creatable = False
Attribute VB_PredeclaredId = False
Attribute VB_Exposed = False
'milk test class
'2001 by Will Raphael and Joel Dobrzelewski
'Michigan State University

Option Explicit

Public calvingDate As Date
Public carCode As String
Public cow As Long
```

```
Public daysInMilk As Double
Public fat As Double
Public milk As Double
Public number As Long
Public protein As Double
Public reason As String

Function fpr() As Double

   If protein <> 0 Then
      fpr = fat / protein
   Else
      fpr = 0
   End If

End Function

Public Function isValid() As Boolean

   'check for out-of-range values for days in milk
   If number = 1 Then
      If daysInMilk < 7 Or daysInMilk > 38 Then
         reason = "test 1 dim=" & daysInMilk
         GoTo NotValid
      End If
   ElseIf number = 2 Then
      If daysInMilk < 32 Or daysInMilk > 75 Then
         reason = "test 2 dim=" & daysInMilk
         GoTo NotValid
      End If

   ElseIf number = 3 Then
      If daysInMilk < 63 Or daysInMilk > 113 Then
         reason = "test 3 dim=" & daysInMilk
         GoTo NotValid
      End If
   End If

   'check for zero/blank values
   If daysInMilk = 0 Or milk = 0 Or fat = 0 Or protein = 0 Then
      reason = "dim milk fat or protein=0"
      GoTo NotValid
   End If

   'check for invalid condition codes
```

```
   If carCode = "AF" Or carCode = "HF" Or carCode = "A" Or carCode = "E" Or
carCode = "F" Or carCode = "I" Or carCode = "L" Then
      reason = "carCode=" & carCode
      GoTo NotValid
   End If

   isValid = True
   Exit Function

NotValid:

   isValid = False

End Function
```

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