# Management parameters from the random regressions testday model to advice farmers on cow nutrition 

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This research was conducted under the auspices of the Graduate School of Wageningen Institute of Animal Sciences (WIAS)

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## Thesis

submitted in fulfillment of the requirements for the degree of doctor<br>at Wageningen University<br>by the authority of the Rector Magnificus<br>Prof. dr. M.J. Kropff,<br>in the presence of the<br>Thesis Committee appointed by the Academic Board<br>to be defended in public<br>on Thursday December 20, 2012<br>at 11 a.m. in the Aula.

Caccamo, M.
Management parameters from the random regressions testday model to advice farmers on cow nutrition.

PhD thesis, Wageningen University, the Netherlands (2012)
With references, with summaries in English and Dutch


#### Abstract

Caccamo, M. (2012). Management parameters from the random regressions testday model to advice farmers on cow nutrition. PhD thesis, Wageningen University, the Netherlands

Accurate monitoring and adequate planning of activities at modern dairy farms are important to improve farm profitability. The aim of this study was to investigate the use of test-day information to support farmers in management of Sicilian dairy herds. To this purpose, a test-day random regression model was developed for the analysis of production data of Sicilian dairy herds. Highest between-herd variation found in the variance components analysis using the test day model showed clear evidence of benefits in using a random regression TD model for management improvement. To identify sources of variation able to explain differences between herds in milk and milk components production herd curves, a field study was conducted in Southern Italy (Ragusa province) where diets and chemical composition of the diet was collected at herd level (every 3 months) and testday milk yield records at individual cow level (every month). Data collection was performed from March 2006 through December 2008 on 40 cooperating farms. Animal breed, feeding system, and total mixed ration chemical composition were identified to influence between-herd variation. At the individual cow level, test day model was further used to investigate the production response to changes in chemical and physical composition of diets in Ragusa province. Starch had the greatest effect on milk, fat, and protein production when crude protein and neutral detergent fiber contents were at a high and low value, respectively. Effects of a nutrition component on production changed when the other nutrients were included in the model, suggesting the confounding response one can have when multiple nutrients are not accounted for.


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## General introduction

### 1.1 INTRODUCTION

The competitiveness of the dairy sector varies between regions in Italy due to the heterogeneity of production structures, types of farms and differences in performance levels. In addition there is considerable diversity with respect to the relationship and the role of dairy farming in relation to the environment. The latter heterogeneity results from differences in natural, historical and cultural conditions that have developed over long period of time. In Sicily approximately 125,000 dairy cattle are raised, with relatively few very large farms because of space limits and a specialization. Dairy cattle in Sicily are raised in concentrated areas of plains and hills of the province of Syracuse, Messina, Palermo and Catania, particularly flourishing in the northwestern part of the province of Ragusa. Compared to Northern regions in Italy, smaller traditional and less modernized systems are present, that have to deal with hot climate, higher costs of feed and energy. The dairy industry in the Hyblean region of Sicily, which mostly consists of the southeastern province of Ragusa, has two main production systems. Modest size herds of dairy cows are generally managed under the traditional system based on pasture and local produced fodder. Milk produced by these cows is mainly used to produce native Ragusano, provola, and ricotta cheeses. Dairy producers on other farms use a more input-intensive, specialized system on larger herds of higher producing Holstein cows, with diets based on total mixed rations. For these herds, milk is produced for fluid and manufacturing purposes. In areas with intensive farming, where attention is paid to genetic improvement and adequate business management based primarily on mechanization and control of environmental parameters, farms are economically competitive.
In the two-year period 2010-2011, total milk production in Sicily was 177,671ton, of which around $62 \%$ was produced in Ragusa province. In this area, 2,767 tons of milk is used to produce Ragusano PDO cheese (year 2008-2009).In the Sicilian region, there are 755 dairies with approved facilities for the processing of milk (data provided by the Regional Health Department) and of these $25 \%$ are located in the province of Ragusa, representing therefore the most important production pole for the dairy sector in Sicily. The prospects for development of the dairy sector in Ragusa are related to adoption of technical and business management practices, cost reduction, meeting the future demand for quality products, implementation of sanitary controls on farms for prevention of animal disease epidemics. The enhancement of quality products is clearly linked to the protection of recognized geographical factors that influence the type of production system within regions.

In 1996, the dairy research center "Consorzio Ricerca Filiera Lattiero-Casearia" (CorFilac) was established to support the development of the dairy sector in Ragusa. The overall goal of CoRFiLaC is to improve the net economic return to dairy farmers and to improve the market competitiveness of Ragusano cheeses for the 300 dairy farms, producing more than $50 \%$ of Sicilian milk. To realize this, CoRFiLaC aims to deliver scientific and technical information to producers in a timely fashion to support management decisions for dairy farms and to support the production of traditional Sicilian dairy products.

## Improving farm management

Management is the decision making process in which limited resources are allocated to a number of production alternatives in such a way that goals and objectives are attained (Kay, 1986). Management is basically described by three main functions (Huirne, 1989): planning, is the systematic design to direct future activities based on available knowledge in order to accomplish the farm's goals and objectives; implementation, is the execution of planned activities; control, involves measuring performance and comparing it with standards (actual of desired). Modifications based on deviations between performance and standards are implemented in the next management cycle. A simplified scheme of farm management cycle is presented in Figure 1.


Figure 1.1 - Farm management cycle. Adapted from (Huirne, 1989).

The information on farm productivity to support management on dairy farms is often collected by Dairy Herd Improvements agencies (DHI). Dairy farmers who are enrolled with a DHI are visited once per month, during a day called "test day". During this visit, the representative collects a large amount of information on the herd such as breeding events (e.g., calving or mating dates) and sex and weight of new-born calves. In addition, the milk production of each cow is measured and a milk sample is collected to determine the fat content, protein content, and somatic cell count. This information is then processed and analyzed centrally (i.e., on DH computers) by taking into account information generated during previous test days at both local and national level. A few days after the test day, the producer receives a report containing the information collected on test day. This report may also contain management information on individual cows and the herd as a whole. The management information provided to the farmers is highly variable from country to country, and sometimes from region to region within the country. The information contained in the report can then be used by the producer for making decisions for the improvement of on-farm management practices. Although DHI data and information can contribute to improved management practices, the benefits only
come about if the farm manager and/or the advisor spend a considerable amount of time in analyzing the incoming information. This process can be time consuming and complex due to the large amount of information. The number of information increases rapidly with number of cows. The analyst needs to combine the DHI information with information which is often not reported in summary sheets, such as feed availability and health status of the herd. To extract all the information to support management decisions is, therefore, not a trivial process. For this reason, it is necessary to develop analytical tools which will accelerate and improve these analyses. Such tools should not only filter and pre-process the data, but also present the results in a way that they are easy to use by the producer. The tool should allow the farmer and/or the advisor to determine rapidly management decisions that will lead to improvement of performance in technical and financial terms.

## Using milk yield in management advice

## Individual cow level

Random regression models that use test-day records for milk yield have been implemented for the estimation of breeding values. The advantage of these models is that (changes in) lactation curves, and variation around typical population or herd lactation curves can be estimated, after adjustment for other factors like age at calving that influence test-day records. Recent studies have investigated the possibility to use a test-day model for management purposes (Koivula et al. 2006, Halasa et al. 2009). Management information is crucial both for accurate monitoring and for adequate planning of activities. The farmer can be supported by advisors in interpreting the information and in making plans. Preferably, interventions (revision of planning) can be applied in an early stage, e.g. before clinical abnormalities have developed. At herd level the average production level is a useful parameter to monitor the overall performance of the herd. At the individual cow level, Interest lies not only in absolute performance level but also in deviations from expected values. The farmer should focus attention on cows with abnormally high or low deviation from expected production, unusual milk composition or high somatic cell counts. As such abnormalities are usually rare this is generally called management by exception (Ouweltjes and De Koning, 2004). The test-day model gives very reliable predictions and is, therefore, very useful to detect potential problem cows. Getting a warning is just a first step. Next is to further diagnose whether it is not a false alarm, and if not what might be the cause
of the problem. For some abnormalities this can be more or less straightforward, but for others more detailed follow-up by the farmer or specialist (e.g. veterinarian) is needed to determine the cause of the abnormality. When the most likely cause is found follow up action is implemented to correct the problem.

## Herd level

Both herd test date effects and herd lactation curves are potentially useful for management support at herd level. Koivula et al. (2006) developed a management tool using a TD model that accounts for month-to-month short-term environmental variation in production through the herd test date classification. Herd test date effects are corrected for environmental and genetic parameters, and are very useful to compare performance of the same herd over time. This management tool can be useful to detect management problems or to determine consequences of changes in herd management: significant changes in feeding, health and other management factors will be reflected in these figures. In order to interpret deviations it is required to have information on the variability of the estimates over time. In The Netherlands, herd specific lactation curves were included in the testday model in order to model differences in lactation curve shape across herds (De Roos et al., 2004). Herd curves were included in order to reduce abnormally high deviations in genetic variation, but have not been considered for management purposes. They can provide information on performance of the entire herd or groups of animals within the herd, especially when they can be compared with a suitable standard. Abnormalities in these curves can indicate specific problems that deserve attention. However, as these figures are calculated on both historical and new information, changes due to herd management factors might appear very slowly. Opportunities to use test-day information at herd level have not been fully exploited.

## The effect of nutrition on lactation curves

It is recognized for a long time that lactation curves are very useful instruments to monitor trends in milk production performance over time (Skidmore et al., 1996) at herd or individual cow level. For proper interpretation of lactation curves, one has to relate information from these curves to management practices and environmental conditions that might impact lactation curves.
At the individual cow level, literature suggests greater production responses to dietary changes are greater in cows with high potential milk production (Brun-

Lafleur et al. 2010). Furthermore, first lactation cows responded differently than multiparous cows to dietary changes. This means that response in milk production to dietary changes is dependent on production potential of the cows and parity. In addition also stage of lactation and relative concentration of dietary nutrients might have an effect. Therefore, a better understanding of the effects of diet on production requires a methodology which takes into account the effects of parity, production potential and lactation stage across multiple diets.
In a study aimed to analyze dairy production needs of cattle owners in Southeastern Sicily, Licitra et al. (1998) observed increasing neutral detergent fiber and decreasing crude protein content of forage as the growing season advanced from early winter to early summer. Forage composition showed drastic undesirable changes in NDF and CP content similar to tropical ecozones, which was correlated with the cause of seasonal effects ( $\mathrm{P}<0.01$ ) on milk production and composition. Abnormal lactation curves (flat without discernible peak, or even convex) were observed especially in spring-calving and in average- or lower-producing cows. A potential cause was the low nutrient intake from reduced forage quality and quantity, resulting in nutrient supplies inadequate to meet requirements. Licitra et al. (1998), in conclusion, suggested a high-priority for Sicily was the need to evaluate forages and other feedstuffs quantitatively in order to optimize forage utilization in better formulated diets for all production system.
Raffrenato et al. (2003) observed differential genetic expression in low and high opportunity Sicilian and Holstein and Brown Swiss herd environments. Low opportunity herds were associated with peakless lactation curves and low frequency of use of nutrition, milking, health, and animal handling. In that study, compression in sire components of variance was translated into diminished genetic gain $(20 \%$ to $60 \%)$ from selection in low compared to high opportunity environments. Environmental constraints were defined as: within herd-year standard deviation for mature equivalent milk yield, detectable incidence of normal vs. abnormal lactation, and causal relationships from high and low frequency use of nutrition, milking, health and animal handling practices. From a management point of view, besides unequal genetic progress caused by decisions altered by diminished daughter milk response and environmental limitations, low producing herds are characterized by low utilization of management practices that prevent adequate expression of genetic potential. This would reinforce the importance of using test day production data to provide farmers in Ragusa province with management advice tools, especially for low producing herds.

### 1.2 OBJECTIVES AND THESIS OUTLINE

The general aim of this thesis is to exploit the opportunities offered by random regression test-day models for milk recording data for the estimation of breeding values and the development of management tools. For this purpose a random regression test-day model was developed, and an experiment was set up that collected information on diet composition on farms along with test-day records of individual cows. The specific objectives of the research in this thesis are 1) to develop the test-day random regression model for the analysis of production data of Sicilian dairy herds, 2) to develop parameters from the random regression model that that can be used to advise dairy farmers on nutritional management of their dairy cows, and 3 ) to investigate the production response to changes in chemical and physical composition of diets in Ragusa province.
The first step was to identify the most important sources of within- and betweenherd variation for the traits and population concerned. For this purpose, variance components for test-day milk, fat and protein yield, and somatic cell score were estimated using a random regression test-day model (Chapter 2). The second step was to develop management parameters that can link differences in parameters of the test-day model to differences in management practices between herds. This variation was investigated by associating animal breed, feeding system, and total mixed ration chemical composition with herd curve traits estimated using a random regression test-day model (Chapter 3).
To assess production response to changes in chemical and physical composition of diets, nutrient composition (Chapter 4) and particle size distribution (Chapter 5) of the total mixed rations were associated with cow lactation curves for milk, fat, and protein yield. Further, the estimates of a single component analysis were compared with a model that takes into account all dietary components simultaneously. In the final chapter, the opportunities to use the information to support management decisions on the individual cows and the herd as a whole are discussed.

## References

Brun-Lafleur, L., L. Delaby, F. Husson, and P. Faverdin. 2010. Predicting energy $\times$ protein interaction on milk yield and milk composition in dairy cows. J. Dairy Sci. 93:4128-4143.
De Roos, A. P. W., A. G. F. Harbers, and G. de Jong. 2004. Random herd curves in a test-day model for milk, fat, and protein production of dairy cattle in the Netherlands. J. Dairy Sci. 87:2693-2701.

Halasa, T., M. Nielen, A.P.W. De Roos, R. Van Hoorne, G. de Jong, T.J.G.M. Lam, T. van Werven, and H. Hogeveen. 2009. Production loss due to new subclinical mastitis in Dutch dairy cows estimated with a test-day model. J. Dairy Sci. 92:599606.

Huirne, R. 1989. Basic concepts of computerized support for farm management decisions. Euro. R. Agr. Eco. 17:69-84.
Kay, R. D. 1986. Farm management. Planning, control, and implementation. New York: McGraw-Hill.

Koivula, M., J. I. Nousiainen, J. Nousiainen, and E. A. Mäntysaari. 2007. Use of herd solutions from a random regression test-day model for diagnostic dairy herd management. J. Dairy Sci. 90:2563-2568.
Licitra, G., R. W. Blake, P. A. Oltenacu, S. Barresi, S. Scuderi, and P. J. Van Soest. 1998. Assessment of the dairy production needs of cattle owners in southeastern Sicily. J. Dairy Sci. 81:2510-2517.
Ouweltjes, W. and Koning, C.A.J.M. de, 2004. Demands and opportunities for operational management support. In: A. Meijering, H. Hogeveen and C.A.J.M. de Koning (editors), Automatic Milking, a better understanding. Wageningen Pers, The Netherlands, p 433-443.
Raffrenato, E., BR. W. Blake, P. A. Oltenacu, J. Carvalheira, G. Licitra. 2003. Genotype by environment interaction for yield and somatic cell score with alternative environmental definitions. J. Dairy Sci. 86:2470-2479.
Skidmore, A. L., A. Brand, and C. J. Sniffen. 1996. Monitoring milk production: decisionmaking and follow-up. Pages 263-281 in Herd health and production management in dairy practice. A. Brand, J.P.T.M. Noordhuizen, Y. H. Schukken, ed. Wageningen Pers, Wageningen, The Netherlands.

## 2

## Variance components for test-day milk, fat, and protein yield, and somatic cell score for analyzing management information

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#### Abstract

Test-day (TD) models are used in most countries to perform national genetic evaluations for dairy cattle. The TD models estimate lactation curves and their changes as well as variation in populations. Although potentially useful, little attention has been given to the application of TD models for management purposes. The potential of the TD model for management use depends on its ability to describe within- or between-herd variation that can be linked to specific management practices. The aim of this study was to estimate variance components for milk yield, milk component yields, and somatic cell score (SCS) of dairy cows in the Ragusa and Vicenza areas of Italy, such that the most relevant sources of variation can be identified for the development of management parameters. The available data set contained $1,080,637$ TD records of 42,817 cows in 471 herds. Variance components were estimated with a multi-lactation, random-regression, TD animal model by using the software adopted by NRS for the Dutch national genetic evaluation. The model comprised 5 fixed effects [region $\times$ parity $\times$ days in milk (DIM), parity $\times$ year of calving $\times$ season of calving $\times$ DIM, parity $\times$ age at calving $\times$ year of calving, parity $\times$ calving interval $\times$ stage of pregnancy, and year of test $\times$ calendar week of test] and random herd $\times$ test date, regressions for herd lactation curve (HCUR), the animal additive genetic effect, and the permanent environmental effect by using fourth-order Legendre polynomials. The HCUR variances for milk and protein yields were highest around the time of peak yield (DIM 50 to 150), whereas for fat yield the HCUR variance was relatively constant throughout first lactation and decreased following the peak around 40 to 90 DIM for lactations 2 and 3 . For SCS, the HCUR variances were relatively small compared with the genetic, permanent environmental, and residual variances. For all the traits except SCS, the variance explained by random herd $\times$ test date was much smaller than the HCUR variance, which indicates that the development of management parameters should focus on between herd parameters during peak lactation for milk and milk components. For SCS, the within-herd variance was greater than the between-herd variance, suggesting that the focus should be on management parameters explaining variances at the cow level. The present study showed clear evidence for the benefits of using a random regression TD model for management decisions.


Key words: dairy cattle, herd management, test-day yield

### 2.1 INTRODUCTION

Test-day (TD) yield records from the milk recording system provide an important source of information for both breeding and management. Herd management improvement and breeding value estimation have been separate processes historically, with different statistical methods and frequencies of data processing. However, there are clear advantages to using the same data and statistical procedures for both management purposes and genetic evaluation.
Test-day models are used in most countries to perform genetic evaluations for dairy cattle by using TD observations instead of aggregated 305-d yield observations (Ptak and Schaeffer, 1993; Reents et al., 1995; Jamrozik and Schaeffer, 1997; Schaeffer et al., 2000). By modeling the shape of the lactation curve and the variability of yields around general shapes, TD models provide 4 to $8 \%$ more accurate genetic evaluations of cows compared with evaluations from 305-d yields (Schaeffer et al., 2000). Random regression TD models are an extension of TD models that allow the shape of the lactation curve to differ for each cow by including random regression coefficients for each animal (Schaeffer and Dekkers, 1994; Jamrozik et al., 1997).
Everett et al. (1994) suggested using the results of TD models for monitoring genetics and management in dairy cattle, and several management applications have been suggested. Mayeres et al. (2004) and Pool and Meuwissen (1999) investigated the ability of a TD model to predict yield from TD records. The inclusion of herd-TD (HTD) and herd curve (HCUR) effects is another important aspect of TD models and would be applicable for management purposes. The HTD effect accounts for month-to-month variability and is particularly informative with regard to short-term management changes that affect the whole herd at a particular TD, for instance, a change in feed ration. Koivula et al. (2007) described the use of monthly herd-management effect solutions from a TD model in Finland. Herd curves, which can be calculated from the random regression TD model, describe differences in lactation curve shapes across herds (De Roos et al., 2004). Those herd-specific lactation curves give information on how animals within a herd perform compared with how they would have done under average management circumstances. Abnormalities in these curves can indicate specific existing problems that deserve extra attention. Variation in lactation peak or persistency across herds can be caused by differences in feeding systems (Horan et al., 2004), daily milking times (Rekik and Ben Gara, 2004), or pregnancy (Tekerli et al., 2000). Therefore, HCUR variance is a good indicator of variability in lactation curve shapes arising from herd management differences.

Given the many options in using the solutions or functions of the solutions of the TD model for interpreting herd management, an important first step is to identify the most important sources of within- and between-herd variation for the traits and population concerned. The second step is to develop management parameters that can link these sources of variation to differences in management practices. The objective of this study was to estimate variance components for TD milk, fat, and protein yield, and SCS by using a random regression TD model. Special focus is given to HCUR and HTD variances, which are mainly related to between- and within-herd management.

### 2.2 MATERIALS AND METHODS

## Data

Test-day milk (kg), fat (g), and protein (g) yield, and somatic cell count (cells/mL) records were available from 2 Italian regions: Ragusa in southeastern Sicily, and Vicenza in the south of Veneto. The records were supplied by CoRFiLaC (Ragusa, Italy) and APA Vicenza (Vicenza, Italy), respectively. In total, there were 4,088,505 TD records of 463,654 lactations of 154,678 cows in 1,303 herds over the period from January 1992 to March 2006. Values for somatic cell count were transformed into somatic cell score (SCS).

Data were edited to extract the more informative records and to ensure connectedness in the data, such as for continuous TD. Data were edited to select: 1) records without missing values; 2) records with pedigree entry available, sire known, and a minimum of 9 daughters per sire; 3) records for 5 to 450 DIM; 4) age at calving in the range mean $\pm 2$ standard deviations; 5) records from HTD with at least 5 TD records; 6) herds with more than 10 HTD; 7) lactations with at least 5 TD records; and 8) lactations with a length of at least 150 d. Data edits 2 through 8 were repeated iteratively until convergence (i.e., until the number of records deleted was negligible).

The resulting data included $2,183,322$ TD records, $53 \%$ of the original. To reduce the memory requirements and computing time, the data set was further reduced by randomly deleting $50 \%$ of herds with animals belonging to the most common, larger breeds (Holstein-Friesian and Brown Swiss). To fulfill the above criteria, the editing procedure was repeated. The final data set used for parameter estimation contained 1,080,637 TD records from 118,580 lactations of 42,817 cows in 471 herds (Table 2.1).

Table 2.1 Data characteristics of the dataset before and after data editing and random selection of $50 \%$ of herds.

|  | Before | After | Range |
| :--- | :---: | :---: | :---: |
| TD records | $4,088,505$ | $1,080,637$ | - |
| Cows | 154,678 | 42,817 | - |
| Herds | 1,303 | 471 | - |
| Test days | 4,487 | 4,126 | - |
| Cows/herd | 120 | 91 | 10 to 999 |
| TD records/cow | 26 | 25 | 5 to 98 |
| Breeds | 11 | 7 | - |

Unknown parents were assigned to 145 phantom pedigree groups based on their selection path ( $\mathrm{SS}=$ sires to breed sons, $\mathrm{SD}=$ sires to breed daughters, $\mathrm{DS}=$ dams to breed sons, and DD = dams to breed daughters), breed, country of origin, and birth year. Small phantom groups were combined within selection path and birth year until reasonable size (>200). The final pedigree was composed of $79.4 \%$ HolsteinFriesian and $15.1 \%$ Brown Swiss, with the remainder being small, crossbred, or unknown breeds.

## Model

Milk, fat, and protein yield, and SCS were analyzed by using a multiple-lactation, single-trait random regression TD model, as described by De Roos et al. (2004):

$$
\begin{aligned}
& y_{i j k l m n o p s t}=\mathrm{rpd}_{\mathrm{i}}+\mathrm{pysd}_{\mathrm{j}}+\text { pay }_{\mathrm{k}}+\mathrm{pcipr}_{\mathrm{l}}+\mathrm{yw}_{\mathrm{m}}+\mathrm{htd}_{\mathrm{n}}+ \\
& +\sum_{\mathrm{q}=0}^{4} \mathrm{z}_{\mathrm{oq}}\left(\mathrm{hc}_{\mathrm{qs}}+\mathrm{ag}_{\mathrm{qst}}+\mathrm{pe}_{\mathrm{qst}}+\left\{\begin{array}{ll}
0, & \text { if } p<3 \\
l s_{p q}, & \text { if } p \geq 3
\end{array}\right\}\right)+e_{\mathrm{ijklmnopst}}
\end{aligned}
$$

where $y_{\mathrm{ijklmn}}{ }^{\text {mpst }}$ is yield record (milk, fat or protein yield, or SCS) of cow $t$ belonging to region $r$ on DIM d of parity $p$ within HTD effect $n$; $r \mathrm{pd}_{\mathrm{i}}$ is region $\times$ parity $\times$ class of DIM class $i(3,470$ classes $)$; pysd $_{j}$ is parity $\times$ year of calving $\times$ season of calving $\times$ class of DIM class $j$ ( 1,656 classes); pay ${ }_{k}$ is parity $\times$ age at calving $\times$ year of calving class $k$ (732 classes); pcipr is parity $\times$ calving interval $\times$ stage of pregnancy class I (153 classes); $\mathrm{yw}_{\mathrm{m}}$ is year of test $\times$ calendar week of test class m ( 312 classes); $h t d_{n}$ is herd $\times$ test date $n\left(49,053\right.$ classes); and $z_{o q}$ is order q Legendre polynomial for DIM o (Kirkpatrick et al., 1990), where o is $\min \{d, 365\}$. In this way TD records with DIM >365 were modeled as DIM = 365 with regard to the regression effects. $\mathrm{hc}_{\mathrm{qs}}$ is the
herd curve effect of herd $\times$ year of test ( 8,007 classes) corresponding to polynomial $q$ of parity $s$, where $s$ is $\min \{p, 3\}$. In this manner, each herd gets a regression curve for parity 1,2 , and $\geq 3$. ag gast is the additive genetic effect of cow t ( 59,882 classes) corresponding to polynomial q of parity s ; $\mathrm{pe}_{\text {qt }}$ is the permanent environmental effect of cow $t$ ( 42,821 classes) corresponding to polynomial $q$ of parity $s$; and $\mathrm{Is}_{\mathrm{pq}}$ is the lactation-specific permanent environmental effect of lactation $p$ corresponding to polynomial q (51,811 classes). Only TD records from lactations with parity $\geq 3$ are assigned to a lactation-specific permanent environmental effect. $\mathrm{e}_{\mathrm{ijklmnopst}}$ is the residual belonging to observation $\mathrm{y}_{\mathrm{ijk} / \mathrm{mnopst}}$.
Residuals were assumed to be uncorrelated between and within animals, with a heterogeneous variance across 27 DIM classes (15-d classes from DIM 5 to 365, plus classes 366 to 390 , 391 to 420 , and 421 to 450 ) within parities $1,2,3,4$, and $\geq 5$. Fourth-order Legendre polynomials were applied to model the random and permanent environmental regression curves.

## Estimation methods

Parameters were estimated by using a Bayesian analysis with Gibbs sampling developed by NRS (De Roos et al., 2004). The algorithm was based on a GaussSeidel iterative best linear unbiased prediction scheme, as described by Janss and De Jong (1999) and was extended to the random regression model by Pool et al. (2000). Uniform priors were assumed for all variance components. Residual variances were sampled from an inverted chi-square distribution, whereas the covariance matrices of the regression coefficients for the HTD, the additive genetic, the permanent environment, the HCUR, and the lactation-specific permanent environment effect were sampled from an inverted Wishart distribution. Burn-in and effective chain length were computed from transition probabilities by using the Gibanal software (Van Kaam, 1998). Estimates of the variance components were calculated as posterior means of the stationary phase of the Gibbs chains.

### 2.3 RESULTS

## Gibbs Chains

Based on the estimated burn-in for all chains and all parameters, a burn-in of 5,000 iterations was chosen for each chain and each parameter. Table 2.2 shows the number of Gibbs chains, the total number of iterations in the chains, and the range in effective chain size across all parameters for milk, fat, protein, and SCS, with 50
being the minimum acceptable number for the effective chain size (Sorensen, 1997).

Table 2.2 Number of Gibbs chains, total number of iterations, and range of effective chain size for the variance component estimation of test-day milk, fat and protein yield and SCS.

| Trait | Milk |  | Fat |  | Protein |  | SCS |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Chains, n | 3 |  | 3 |  | 3 |  | 3 |  |
| Iterations, n (total) | 328,780 |  | 346,400 |  | 470,120 |  | 403,500 |  |
| Iterations, n (excl. burn-in) | 253,780 |  | 271,400 |  | 395,120 |  | 328,500 |  |
| Range in effective chain size | Min | Max | Min | Max | Min | Max | Min | Max |
| Additive genetic | 203 | 510 | 206 | 554 | 314 | 653 | 240 | 609 |
| Permanent environment | 58 | 627 | 66 | 700 | 72 | 782 | 50 | 801 |
| Lactation-specific permanent environment | 938 | 10,940 | 449 | 16,341 | 1,111 | 15,652 | 709 | 13,441 |
| Herd curve | 455 | 1,819 | 310 | 2,645 | 445 | 3,674 | 253 | 2,797 |
| Herd $\times$ test date | 817 | 817 | 441 | 441 | 1,196 | 1,196 | 363 | 363 |
| Residual | 981 | 32,867 | 712 | 34,631 | 1,136 | 47,002 | 796 | 40,343 |

## TD Variance Components

The estimated additive genetic, permanent environment, lactation-specific permanent environment, HTD, HCUR, and residual variances in lactations 1, 2, and 3 for TD milk, fat, and protein yields, and SCS are given in Figures 2.1, 2.2, 2.3, and 2.4, respectively.

For all traits, except SCS, additive genetic variances increased slowly during the lactation trajectory in all 3 parities. For milk and protein yields, the residual variance was relatively small compared with the total phenotypic variance, indicating a good fit of the model. The residual variance was larger for fat yield and largest for SCS, indicating that the model could explain less variance and that observations for these traits might therefore be less predictable.


Figure 2.1 Additive genetic (GEN, $\uparrow$ ), permanent environmental (PERM, $■$ ), lactation specific permanent environmental (LSPE, $\square$ ), herd $\times$ test date (HTD, $\times$ ), herd curve (HCUR, +), and residual (RES, $৩$ ) variance of test-day milk yield in lactations 1,2 , and 3 (in $\mathrm{kg}^{2}$ ).


Figure 2.2 Additive genetic (GEN, $\uparrow$ ), permanent environmental (PERM, $■$ ), lactation specific permanent environmental (LSPE, $\square$ ), herd $\times$ test date (HTD, $\times$ ), herd curve (HCUR, +), and residual (RES, $仓$ ) variance of test-day fat yield in lactation 1,2 and 3 (in $\mathrm{g}^{2}$ ).


Figure 2.3 Additive genetic (GEN, $\uparrow$ ), permanent environmental (PERM, ■), lactation specific permanent environmental (LSPE, $\square$ ), herd $\times$ test date (HTD, $\times$ ), herd curve (HCUR, +), and residual (RES, 0 ) variance of test-day protein yield in lactation 1,2 and 3 (in $\mathrm{g}^{2}$ ).


Figure 2.4 Additive genetic (GEN, $\uparrow$ ), permanent environmental (PERM, ■), lactation specific permanent environmental (LSPE, ם), herd $\times$ test date (HTD, $\times$ ), herd curve (HCUR, +), and residual (RES, $\rangle$ ) variance of test-day SCS in lactation 1,2 and 3.

Herd $\times$ test date variances for milk and milk components yields were much lower than HCUR variances, which indicates that differences between herds are larger than differences between test dates within a herd. Around peak yield (DIM 50 to 150), HCUR variances were greatest for milk and protein yield, whereas for SCS, HCUR variances were relatively small compared with the other variance components. This result indicates relatively small differences between herds for SCS.
In Figure 2.5, the estimated ratio of HCUR over phenotypic variances in lactations 1, 2, and 3 across DIM is shown for TD milk, fat, and protein yields, and SCS. For all traits except SCS, the ratio of HCUR to phenotypic variances peaked at around 50 to 150 DIM and decreased at the end of the lactation to approximately 0.15 , except for the first lactation, which did not decrease below 0.35 . The greatest HCUR over phenotypic variances were observed for protein yield.


Figure 2.5 Ratio of the herd curve over phenotypic variance in lactation 1, 2 and 3 for testday milk ( $\mathbf{\square}$ ), fat ( $\uparrow$ ) and protein ( $\square$ ) yield and SCS ( 0 ).

Table 2.3 gives HCUR correlations among DIM 5, 65, 185, 305, and 365 for parities 1,2 , and 3. For all traits, HCUR correlations among DIM for parities 2 and 3 were similar, and both were higher than during parity 1. Overall correlations for all parities for milk, fat, and protein yields were high, ranging from 0.69 to 0.99 . On the other hand, correlations for SCS among DIM were low, with the lowest values for DIM 5 in the first parity.

| Trait | DIM | Lactation 1 |  |  |  | Lactation 2 |  |  |  | Lactation 3 |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | DIM 65 | DIM 185 | DIM 305 | DIM 365 | DIM 65 | DIM 185 | DIM 305 | DIM 365 | DIM 65 DIM 185 |  | DIM 305 | DIM 365 |
| Milk | 5 | 0.78 | 0.71 | 0.69 | 0.69 | 0.92 | 0.90 | 0.88 | 0.85 | 0.87 | 0.85 | 0.85 | 0.84 |
|  | 65 |  | 0.98 | 0.95 | 0.92 |  | 0.99 | 0.95 | 0.88 |  | 0.99 | 0.96 | 0.90 |
|  | 185 |  |  | 0.99 | 0.97 |  |  | 0.98 | 0.91 |  |  | 0.98 | 0.93 |
|  | 305 |  |  |  | 0.99 |  |  |  | 0.96 |  |  |  | 0.97 |
| Fat | 5 | 0.81 | 0.712 | 0.67 | 0.65 | 0.92 | 0.87 | 0.82 | 0.78 | 0.90 | 0.86 | 0.82 | 0.81 |
|  | 65 |  | 0.97 | 0.93 | 0.89 |  | 0.98 | 0.92 | 0.85 |  | 0.98 | 0.93 | 0.88 |
|  | 185 |  |  | 0.98 | 0.96 |  |  | 0.97 | 0.91 |  |  | 0.98 | 0.92 |
|  | 305 |  |  |  | 0.99 |  |  |  | 0.97 |  |  |  | 0.97 |
| Protein | 5 | 0.80 | 0.75 | 0.73 | 0.74 | 0.93 | 0.92 | 0.89 | 0.86 | 0.88 | 0.86 | 0.86 | 0.86 |
|  | 65 |  | 0.98 | 0.96 | 0.94 |  | 0.99 | 0.95 | 0.88 |  | 0.99 | 0.96 | 0.91 |
|  | 185 |  |  | 0.99 | 0.98 |  |  | 0.98 | 0.92 |  |  | 0.98 | 0.94 |
|  | 305 |  |  |  | 0.99 |  |  |  | 0.97 |  |  |  | 0.98 |
| SCS | 5 | 0.36 | 0.31 | 0.34 | 0.31 | 0.69 | 0.59 | 0.49 | 0.53 | 0.74 | 0.63 | 0.60 | 0.54 |
|  | 65 |  | 0.84 | 0.86 | 0.80 |  | 0.89 | 0.80 | 0.74 |  | 0.92 | 0.89 | 0.81 |
|  | 185 |  |  | 0.96 | 0.95 |  |  | 0.94 | 0.90 |  |  | 0.96 | 0.92 |
|  | 305 |  |  |  | 0.96 |  |  |  | 0.95 |  |  |  | 0.95 |

Herd curves for second-parity protein yield for the 10 largest herds from Vicenza and the 10 largest herds from Ragusa are shown in Figure 2.6. It is surprising to see why there were such large differences in variation between the regions.


Figure 2.6 Herd curve for protein yield in second lactation of the 10 largest herds of Vicenza (—) and the 10 largest herds of Ragusa (-1-1.).

### 2.4 DISCUSSION

Variance components of TD milk, fat, and protein yields, and SCS were estimated by applying a random regression animal model to a large data set with records of dairy cows from the Ragusa and Vicenza areas of Italy. Estimated additive genetic, permanent environmental, and residual variances are in line with other studies (De Roos et al., 2004; Gengler et al., 2004).
The HCUR variances were highest around peak yield (DIM 50 to 150) for all traits except SCS. This is in contrast with the variances found by De Roos et al. (2004) and Gengler and Wiggans (2001). In those studies, variance of the random herd curves was greatest at the borders of the lactation and negligible in midlactation. This is surprising, and is probably an artifact of the missing data and the statistical model used to extrapolate the data (Pool and Meuwissen, 1999). Herd lactation curves are deviations from overall curves and can be compared between herds. They indicate how the animals in the herd perform compared with how they would have done under average management circumstances. Greater variability at the peak
indicates that differences in management between herds are expected to have the largest impact around the peak of the lactation. The ratio of HCUR over phenotypic variance can be interpreted as the ratio between across-herd and across-animal variation. This ratio was greatest for protein yield around the time of peak yield, with values greater than 1 for the first and second lactation, showing that variability between herds is greater than variability between animals. For this reason, it could be argued that development of management parameters for milk, fat, and protein yields around the peak should focus on between-herd parameters rather than management parameters that compare individual cows. Therefore, greater HCUR variances represent a promising opportunity for management improvement between herds. As an example, positive herd curves for milk, fat, and protein and negative herd curves for SCS could indicate that herd management is better than average. Negative lactation herd curve traits (peak, mean, and persistency) are highly correlated with low-energy diets and low starch content in feeds. Because these curves are estimated for successive years and are not primarily based on the most recent data, they indicate mid- to long-term management effects.
The HCUR variability is even more extreme if we compare HCUR vs. HTD variance, namely, between- vs. within-herd variation. Herd-TD is defined as a deviation from the mean within each herd. Therefore, HTD estimates are not useful for comparing farms. The HTD effect is especially informative for immediate management changes that affect the whole herd at a precise TD. In particular, negative milk, fat, and protein deviations and positive SCS deviations indicate that cows produced less milk, fat, and protein and more cells than expected. A sudden drop in milk and fat content yield at a particular test day could alert managers of insufficient effective fiber in feeds that could lead to acidosis at the herd level. On the other hand, an increase in fat content combined with a drop in milk and protein yield could alert managers of an energy unbalance in the diets that could lead to ketosis. Positive SCS deviations could be due to malfunctions of the milking system or to infectious diseases. Higher variability in HCUR rather than an HTD effect would suggest that the focus should be on management parameters that describe between-herd variation; consequently, advice is needed mostly for long-term rather than shortterm changes.
For SCS, both HCUR and HTD variances were relatively small compared with the other variance components. This would suggest that the focus should be on management parameters that describe between animal variation; consequently, management considerations are needed mostly at the cow level for this trait. The HCUR correlations for SCS among DIM were also lower than for other traits and
were very low between 5 and greater DIM, meaning that management practices affecting early lactation do not have a directly related impact on somatic cell count later on in the same lactation.

The ratio of HCUR over phenotypic variance was highest for protein yield in the second lactation. The estimated herd curves of the 10 largest herds from Ragusa and the 10 largest herds from Vicenza for protein yield in the second lactation shown in Figure 2.6 clearly reveal much greater variation in the shape of the lactation curve as well as in the deviation from the lactation curve of the population for Ragusa than for Vicenza province. This greater variation is not simply caused by differences in the mean region effects that were included in the model as a fixed effect. A more likely option might be the lower and more variable feed quality in the Ragusa region, leading to more variation between herds. Further work will focus on identifying the sources of variation in these random herd curves across herds, including the measured feed quality at the herd level.
Test-day variance components estimated in the present study showed clear evidence of the benefits of using a random regression TD model for management improvement, by improving both between- and within-herd management aspects.

## Acknowledgments

The authors thank J. van Arendonk and J. D. Ferguson for their scientific support. This research was funded in the "Accordo di Programma Quadro" action from the Assessorato Industria of the Sicilian Region (Palermo, Italy).

## REFERENCES

De Roos, A. P. W., A. G. F. Harbers, and G. de Jong. 2004. Random herd curves in a test-day model for milk, fat, and protein production of dairy cattle in the Netherlands. J. Dairy Sci. 87:2693-2701.

Everett, R. W., F. Schmidt, and L. H. Wadell. 1994. A test-day model for monitoring management and genetics in dairy cattle. J. Dairy Sci. 77(Suppl. 1):267. (Abstr.)
Gengler, N., and G. R. Wiggans. 2001. Variance of effects of lactation stage within herd by herd yield. J. Dairy Sci. 84(Suppl. 1):216. (Abstr.)
Gengler, N., G. R. Wiggans, and A. Gillon. 2004. Estimated heterogeneity of phenotypic variance of test-day yield with a structural variance model. J. Dairy Sci. 87:1908-1916.

Horan, B., P. Dillon, D. P. Berry, P. O'Connor, and M. Rath. 2004. The effect of strain of Holstein-Friesian, feeding system and parity on lactation curves characteristics of spring-calving dairy cows. Livest. Prod. Sci. 95:231-241.

Jamrozik, J., and L.R. Schaeffer. 1997. Estimates of genetic parameters for a test day model with random regressions for yield traits of first lactation Holsteins. J. Dairy Sci. 80:762-770.
Jamrozik, J., L. R. Schaeffer, and J. C. M. Dekkers. 1997. Genetic evaluation of dairy cattle using test day yields and random regression model. J. Dairy Sci. 80:12171226.

Janss, L. L. G., and G. De Jong. 1999. MCMC based estimation of variance components in a very large dairy cattle data set. Pages 63-68 in Interbull Bull. No. 20. Interbull, Tuusula, Finland.
Kirkpatrick, M., D. Lofsvold, and M. Bulmer. 1990. Analysis of the inheritance, selection and evolution of growth trajectories. Genetics 124:979-993.
Koivula, M., J. I. Nousiainen, J. Nousiainen, and E. A. Ma"ntysaari. 2007. Use of herd solutions from a random regression test-day model for diagnostic dairy herd management. J. Dairy Sci. 90:2563-2568.
Mayeres, P., J. Stoll, J. Bormann, R. Reents, and N. Gengler. 2004. Prediction of dailymilk, fat, and protein production by a random regression test-day model. J. Dairy Sci. 87:1925-1933.
Pool, M. H., L. L. G. Janss, and T. H. E. Meuwissen. 2000. Genetic parameters of Legendre polynomials for first parity lactation curves. J. Dairy Sci. 83:2640-2649.
Pool, M. H., and T. H. E. Meuwissen. 1999. Prediction of daily milk yields from a limited number of test days using test day models. J. Dairy Sci. 82:1555-1564.
Ptak, E., and L. R. Schaeffer. 1993. Use of test day yields for genetic evaluation of dairy sires and cows. Livest. Prod. Sci. 34:23-34.
Reents, R., J. C. M. Dekkers, and L. R. Schaeffer. 1995. Genetic evaluation for somatic cell score with a test day model for multiple lactations. J. Dairy Sci. 78:2858-2870.
Rekik, B., and A. Ben Gara. 2004. Factors affecting the occurrence of atypical lactations for Holstein-Friesian cows. Livest. Prod. Sci. 87:245-250.
Schaeffer, L. R., and J. C. M. Dekkers. 1994. Random regressions in animal models for test-day production in dairy cattle. Page 443 in Proc. 5th World Congr. Genet. Appl. Livest. Prod., Guelph, Ontario, Canada.
Schaeffer, L. R., J. Jamrozik, G. J. Kistemaker, and B. J. Van Doormaal. 2000. Experience with a test-day model. J. Dairy Sci. 83:1135-1144.
Sorensen,D. 1997. Gibbs sampling in quantitative genetics. Internal Report No. 82. Danish Inst. Anim. Sci., Res. Ctr. Foulum, Tjele, Denmark.
Tekerli, M., Z. Akinci, I. Dogan, and A. Akcan. 2000. Factors affecting the shape of lactation curves of Holstein cows from the Balikesir province of Turkey. J. Dairy Sci. 83:1381-1386.

Van Kaam, J. B. C. H. M. 1998. Gibanal 2.9. Analyzing Program for Markov Chain Monte Carlo Sequences. Dept. Anim. Sci., Wageningen Agric. Univ., Wageningen, the Netherlands.

## 3

## Associations of breed and feeding management with milk production curves at herd level using a random regression test-day model

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#### Abstract

Earlier studies identified large between-herd variation in estimated lactation curve parameters from test-day milk yield and milk composition records collected in Ragusa province, Italy. The objective of this study was to identify sources of variation able to explain these between-herd differences in milk production curves, by estimating associations of animal breed (Holstein Friesian vs. Brown Swiss), feeding system [separate feeding vs. total mixed ration (TMR)], and TMR chemical composition on milk and milk components herd curves. Data recorded from 1992 through 2007 for test-day (TD) milk, fat, and protein yields from 1,287,019 records of 148,951 lactations of 51,489 cows in 427 herds were processed using a random regression TD model. Random herd curves (HCUR) for milk, fat, and protein yields were estimated from the model per herd, year, and parity (1, 2, and 3+) using 4order Legendre polynomials. From March 2006 through December 2007, samples of TMR were collected every 3 mo from 37 farms in Ragusa province. Samples were analyzed for dry matter, ash, crude protein, soluble nitrogen, acid detergent lignin, neutral detergent fiber, acid detergent fiber, and starch. Traits used to describe milk production curves were peak, days in milk at peak, persistency, and mean. Association of feeding system and animal breed with HCUR traits was investigated using a general mixed model procedure. Association of TMR chemical composition with HCUR traits was investigated using multivariate analysis with regression and stepwise model selection. Results were consistent for all traits and parities. Feeding system was significantly associated with HCUR peak and mean, with higher values for TMR. Animal breed was significantly associated with HCUR persistency, with higher values for Brown Swiss herds. Furthermore, animal breed influenced HCUR peak and mean, with higher values for Holstein Friesian herds. Crude protein had the largest effect on HCUR peak and mean, whereas the interaction between crude protein and dry matter mainly affected persistency. When provided by a national evaluation system, HCUR can be used as an indicator of herd feeding management.


Key words: herd curve, feeding management , test-day model

### 3.1 INTRODUCTION

Test-day (TD) models are used in most countries to perform genetic evaluations of milk production for dairy cattle by using TD observations instead of aggregated 305-d yield observations (Ptak and Schaeffer, 1993; Reents et al., 1995; Jamrozik et al., 1997a; Schaeffer et al., 2000). Because a milk recording system is also an important source of information for management, clear advantages have been reported by several authors to use the same data and statistical procedures for management purposes and genetic evaluation. Everett et al. (1994) suggested using results of TD models for monitoring genetics and management in dairy cattle. Several management applications have been suggested. Mayeres et al. (2004) and Pool and Meuwissen (1999) investigated the ability of a TD model to predict yield from TD records, where information from national evaluation systems provided information for individual farmers. Herd curves were included in the TD model used for routine evaluation in the Netherlands to adjust abnormal additive genetic variance at the extremes of lactations (De Roos et al., 2004). Caccamo et al. (2008) investigated the possibility of using random regression TD model (RRTDM) outputs to give advice about farm management, and these authors found that random regression herd curves differed remarkably between herds. Their results showed that herd curve variance for dairy cattle in Ragusa and Vicenza provinces was extremely high for milk, fat, and protein yields, especially at the peak of the lactation, suggesting that variation could be explained by differences in management practices across herds that mainly influence peak production.
The advantages of using total mixed ration (TMR) instead of separate feeding include eliminating choice among feeds, higher production, reduced digestive upsets early in lactation, non-protein nitrogen fed multiple times with other feed ingredients throughout the day, reduced labor, prevention of milk fat depression by providing a specific forage-to-concentrate ratio, quantitative formulation of the diet, and mechanization of the feeding procedure (Coppock, 1977). Several studies have investigated the effect of using different feeding strategies on milk production: some reported higher production when feeding TMR compared with separate feeding (Gordon et al., 1995; Bargo et al., 2002), whereas others found no (or very small) effects (Gordon et al., 1995; Yrjänen et al., 2003; Ferris et al., 2006). The objective of this study was to identify sources of variation able to explain differences between herds in milk and milk component production curves obtained from routine evaluation software. This variation was explained by associating animal breed, feeding system, and TMR chemical composition with herd curve traits estimated using a RRTDM.

### 3.2 MATERIALS AND METHODS

## Data

To estimate random curves, a TD model on a full data set was run using a software developed initially for the Dutch national genetic evaluation. Data were supplied by the local milk-recording agency (APA, Ragusa, Italy) and included 1,287,019 TD records of milk (kg), fat (g), and protein (g) of 148,951 lactations and collected on 51,489 cows in 427 herds from January 1992 to April 2008.
For a subset of these farms and years, information on feeding practices was collected every 3 mo from 40 farms in Ragusa province in Southern Italy from March 2006 through December 2007. Selection of these farms was based on farmers' agreement to participate in the study and cooperate with data collection requirements and based on a convenience sample of feeding system [separate feeding (SF) vs. TMR], and breed of the animals (Holstein Friesian vs. Brown Swiss; Table 3.1). During data collection, 2 farms withdrew and 1 farm changed feeding system from SF to TMR. Therefore, those 3 farms were excluded from data analysis. Out of the remaining farms, 28 ( 6 Brown Swiss and 22 Holstein Friesian) fed their animals using a TMR system, whereas 9 farms ( 3 Brown Swiss and 6 Holstein Friesian) used a traditional SF system. Samples of TMR were analyzed for dry matter (DM) at $100^{\circ} \mathrm{C}$ (AOAC, 1994), ash (AOAC, 1994), crude protein (CP, AOAC, 1994), soluble nitrogen (SN, Licitra et al., 1996), acid detergent lignin (ADL, Goering and Van Soest, 1970), neutral detergent fiber (NDF, Van Soest et al., 1991), acid detergent fiber (ADF, Goering and Van Soest, 1970), and starch (AOAC 1998; method 996.11). All chemical analyses were expressed on a DM basis. For both feeding strategies, diets were evaluated using CPM Dairy (version 3.0.8, Cornell University, Ithaca, NY; University of Pennsylvania, Kennett Square, PA; and Miner Agricultural Research Institute, Chazy, NY).

Table 3.1 Distribution of 37 herds per feeding system (TMR vs. separate feeding) and animal breed (Holstein Friesian vs. Brown Swiss).

|  |  | Cows (n) |  |  |  |
| :--- | :---: | :---: | :---: | :---: | :---: |
|  | Herds (n) | Mean | SD | Minimum | Maximum |
| TMR |  |  |  |  |  |
| Holstein Friesian | 22 | 70.2 | 35.7 | 22 | 157 |
| Brown Swiss | 6 | 43.2 | 11.8 | 25 | 57 |
| Subtotal | 28 | 64.4 | 33.8 | 22 | 157 |
|  |  |  |  |  |  |
| Separate feeding |  |  |  |  | 48 |
| Holstein Friesian | 6 | 28.5 | 10.6 | 20 | 48 |
| Brown Swiss | 3 | 24.7 | 8.1 | 19 | 48 |
| Subtotal | 9 | 27.2 | 9.5 | 19 |  |
|  |  |  |  |  | 157 |

## Estimation of Herd Curve Traits

Production TD records for the full data set were processed using a multiplelactation, single-trait RRTDM. The software and model were adapted to this study from the model used by NRS (Arnhem, the Netherlands) for the Dutch national genetic evaluation (NRS, 2009), as described by Caccamo et al. (2008):

$$
\begin{aligned}
& y_{\text {dijklmnopr }}=\mathrm{pd}+\mathrm{pysq}+\mathrm{pa}_{\mathrm{k}}+\mathrm{pcipr}+\mathrm{yw}_{\mathrm{m}}+\mathrm{htc}_{\mathrm{h}}+ \\
& +\sum_{\mathrm{q}=0}^{4} \mathrm{z}_{\mathrm{oq}}\left(\text { hcu }_{\mathrm{q}}+\mathrm{ag}_{\mathrm{qrs}}+\mathrm{pe}_{\text {qrs }}+\left\{\begin{array}{ll}
0, & \text { ifp }<3 \\
s_{\mathrm{pq},}, & \text { ifp } \geq 3
\end{array}\right\}\right)+\mathrm{e}_{\text {dijklmnopr }}
\end{aligned}
$$

where $y_{\text {dijklmnoprs }}=$ yield record (milk, fat or protein yield, or SCS) of the cow s on DIM d of parity $p$ within herd test-day (HTD) effect $n$ and belonging to fixed effect class $\mathrm{i}, \mathrm{j}, \mathrm{k}, \mathrm{I}$, and m defined as follows; $\mathrm{pd}_{\mathrm{i}}=$ ith class of parity ( 7 levels) $\times$ class of DIM ( 2,695 classes; 385 DIM classes); pysd $_{j}=$ jth class of parity $\times$ year of calving $\times$ season of calving $\times$ class of DIM (1,680 classes; 14 DIM classes); pay $_{k}=k$ th class of parity $\times$ age at calving $\times$ year of calving ( 368 classes); pcipr $_{\text {I }}=1$ lh class of parity $\times$ calving interval $\times$ stage of pregnancy ( 270 classes); $y_{m}=m$ th class of year of test $\times$
calendar week of test ( 260 classes); htd $\mathrm{n}_{\mathrm{n}}=$ random herd $\times$ test date $\mathrm{n}(42,481$ classes); $\mathrm{z}_{\mathrm{oq}}=$ order $q$ Legendre polynomial for DIM o (Kirkpatrick et al., 1990), where $o=\min \{d, 365\}$. In this way, TD records with DIM >365 were modeled as DIM $=365$ with regard to the regression effects; hcur $_{\text {qr }}=$ random herd curve (HCUR) effect of herd $\times$ year of test (4,094 classes) corresponding to polynomial q of parity $r$, where $r=\min \{p, 3\}$. In this manner, each herd gets a regression curve for parity 1 , 2 and $\geq 3$; ag $_{q r s}=$ random additive genetic effect of cow $s$ ( 59,977 classes) corresponding to polynomial $q$ of parity $r$; $\mathrm{pe}_{\mathrm{qrs}}=$ random permanent environmental effect of cow s (51,489 classes) corresponding to polynomial q of parity $r$; $\mathrm{Is}_{\mathrm{pq}}=$ lactation-specific permanent environmental effect of lactation p corresponding to polynomial q (76,571 classes). Only TD records from lactations with parity $\geq 3$ are assigned to a lactation specific permanent environmental effect. In this manner, lactations with parity $\geq 3$ have one common permanent environmental curve and one specific curve for each lactation; $\mathrm{e}_{\text {dijklmnoprs }}=$ residual belonging to observation $\mathrm{y}_{\text {dijklmnoprs }}$.
Unknown parents were assigned to 259 phantom pedigree groups based on their selection path (sires to breed sons, sires to breed daughters, dams to breed sons, and dams to breed daughters), breed, country of origin, and birth year. Random effects were HTD and HCUR, animal additive genetic effect, and permanent environmental effect modeled using fourth-order Legendre polynomials. The random and permanent environmental regression curves were modeled using fourth-order Legendre polynomials.
Fixed and random effects included in the model run by NRS for the national genetic evaluation were adapted to the data and the aim of this study. In particular, differences occur in the inclusion of 2 fixed effects (parity $\times$ age at calving $\times$ year $\times$ season of calving and parity $\times$ age at calving $\times$ year $\times$ season of calving $\times$ lactation stage), that were replaced by pay and pysd, respectively. The fixed effect number of days dry $\times$ lactation stage was removed from the NRS model and yw was added in this study, whereas HTD was included as a random effect. Parities used in this study were 7, whereas in NRS model only the first 3 parities are used. The decision to include all parities in the model implementation described in this study was primarily dictated by the need to use TD estimates coming out from the model for all the cows for management purposes.

The traits used to describe HCUR were peak, DIM at peak (DIMP), persistency, and mean. Depending on the shape of the lactation curve (convex or concave), the peak was estimated as the maximum or the minimum of the curve respectively when it does not occur at the beginning or at the end of the lactation.

Persistency (P) was defined as

$$
P=\frac{1}{245} \sum_{\mathrm{i}=61}^{305} \mathrm{y}_{\mathrm{i}}-\mathrm{y}_{60}
$$

(Kistemaker, 2003), where $\mathrm{y}_{\mathrm{i}}=$ yield at DIM i. Herd curve traits were estimated per year during the study for parities 1,2 , and $3+$ for each of the 37 farms involved in data collection.

## Statistical Analysis

To identify the variation sources, HCUR traits were associated with breed and feeding system variables and TMR chemical components. The first analysis included the traits of the curves estimated for each herd, parity, and year when management information was collected (2006 and 2007), the feeding system (TMR vs. SF), and the breed (Holstein Friesian vs. Brown Swiss). Using the data set with 37 herds, association of feeding system and breed with HCUR traits was investigated using SAS MIXED procedure (version 9.1.3, SAS Institute Inc., Cary, NC) applied to a linear mixed model having each curve trait (peak, DIMP, persistency, and mean) per herd, parity $(1,2$, or 3$)$, and year $(2006,2007)$ as dependent variables. Breed, feeding system, year, and their interactions were included in the model as fixed effects, whereas farm within breed was treated as a repeated observation. Means for breed and feeding system were tested using pairwise Ismeans coupled with Bonferroni's adjustment.
The second analysis included the traits of the curves estimated for the 28 herds with TMR for each year (2006 and 2007) and the yearly average of the detailed chemical composition of the TMR. Association of composition of the TMR composition with the shape of lactation curve was investigated using the following linear regression model:
$\mathrm{t}_{\mathrm{ijk}}=\mu+\mathrm{DM}_{\mathrm{ijk}}+\mathrm{ASH}_{\mathrm{ijk}}+\mathrm{CP}_{\mathrm{ijk}}+\mathrm{SN}_{\mathrm{ijk}}+\mathrm{ADL}_{\mathrm{ijk}}+\mathrm{NDF}_{\mathrm{ijk}}+\mathrm{ADF}_{\mathrm{ijk}}+\mathrm{STARCH}_{\mathrm{ijk}}+\mathrm{e}_{\mathrm{ijk}}$
where $\mathrm{t}_{\mathrm{ijk}}$ is the curve trait (peak, DIMP, persistency, and mean) of the ith herd for the jth parity in the kth year (2006, 2007); $\mathrm{DM}_{\mathrm{ijk}}, \mathrm{Ash}_{\mathrm{ijk}}, \mathrm{CP}_{\mathrm{ijk}}$, $\mathrm{SN}_{\mathrm{ijk}}$, $\mathrm{ADL}_{\mathrm{ijk}}$, $\mathrm{NDF}_{\mathrm{ijk}}$, $\mathrm{ADF}_{\mathrm{ijk}}$, and Starch $_{\mathrm{ijk}}$ are the average chemical composition of TMR sampled for the ith herd and jth parity within kth year. Interactions were also included in the model but only reported when significant.

To select the subset of independent variables that best explain each dependent variable and to avoid inclusion of regressors correlated to one another, the backward-forward elimination, stepwise selection option was used in PROC REG, multi-regression procedure using SAS statistical software (SAS Institute Inc.). The default significance level of 0.15 was used for the variables to enter in and remain in the model as other variables entered the model.

### 3.3 RESULTS

Least squares means values for peak, DIMP, persistency, and mean for milk, fat, and protein HCUR, grouped by feeding system and by breed are presented in Table 3.2. Feeding system had the largest effect ( $P<0.05$ ) on peak and mean for fat and protein yields for all parities. Herds using a TMR had higher peak values compared with those using SF for fat HCUR ( $0.23,0.25$, and 0.28 vs. $0.03,0.11$, and 0.12 g for parities 1,2 , and $3+$, respectively) and protein $\operatorname{HCUR}$ ( $0.18,0.22$, and 0.22 vs. 0.08 , 0.11 , and 0.11 g for parities 1,2 , and $3+$, respectively).

Animal breed affected peak and mean for milk yield and persistency for all traits and parities 2 and 3+. Holstein Friesian herds had higher values compared with Brown Swiss herds for milk HCUR peak (5.32, 6.69, and 6.96 vs. $2.83,3.26$, and 3.62 kg for parities 1, 2, and 3+, respectively) and milk HCUR mean (4.63, 5.41, and 5.41 vs. $2.50,2.96$, and 3.00 kg for parities 1,2 , and $3+$, respectively). Brown Swiss herds had higher persistency values compared with Holstein Friesian herds for milk HCUR ( -0.17 and -0.23 vs. -1.21 and -1.21 kg for parities 2 and $3+$, respectively), fat HCUR (0.02, -0.01 , and -0.02 vs . $0.00,-0.05$, and -0.05 g for parities 1,2 , and $3+$, respectively), and protein HCUR ( 0.00 vs. -0.02 g for both parities 2 and $3+$ ). A significant effect of the interaction between animal breed and feeding system was found only for DIMP in first lactation milk HCUR.

Table 3.2 Least squares means values of peak, DIM at peak, persistency, and mean of herd curves for milk (kg), fat (g), and protein (g) ${ }^{1}$

| Curve Trait | Parity | Feeding system |  | Animal Breed |  |
| :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | Traditional | TMR | Brown <br> Swiss | Holstein <br> Friesian |
| Peak |  |  |  |  |  |
| Milk | 1 | $2.57{ }^{\text {b }}$ | $5.25{ }^{\text {a }}$ | $2.42{ }^{\text {b }}$ | $5.41{ }^{\text {a }}$ |
|  | 2 | $2.57{ }^{\text {b }}$ | $6.75{ }^{\text {a }}$ | $2.56{ }^{\text {b }}$ | $6.76{ }^{\text {a }}$ |
|  | $3+$ | $3.18{ }^{\text {b }}$ | $7.01{ }^{\text {a }}$ | $3.15{ }^{\text {b }}$ | $7.04{ }^{\text {a }}$ |
| Fat | 1 | $0.02{ }^{\text {b }}$ | $0.24{ }^{\text {a }}$ | 0.09 | 0.17 |
|  | 2 | $0.07{ }^{\text {b }}$ | $0.26{ }^{\text {a }}$ | 0.14 | 0.19 |
|  | $3+$ | $0.08{ }^{\text {b }}$ | $0.28{ }^{\text {a }}$ | 0.15 | 0.21 |
| Protein | 1 | $0.05{ }^{\text {b }}$ | $0.19^{\text {a }}$ | 0.08 | 0.16 |
|  | 2 | $0.09{ }^{\text {b }}$ | $0.23{ }^{\text {a }}$ | 0.11 | 0.20 |
|  | $3+$ | $0.09{ }^{\text {b }}$ | $0.23{ }^{\text {a }}$ | 0.12 | 0.21 |
| DIM at Peak |  |  |  |  |  |
| Milk | 1 | 137.92 | 131.81 | 124.02 | $145.70^{2}$ |
|  | 2 | 89.67 | 93.19 | 98.93 | 83.93 |
|  | $3+$ | 93.25 | 106.63 | 105.00 | 94.88 |
| Fat | 1 | 141.25 | 157.25 | 174.50 | 124.00 |
|  | 2 | 131.13 | 112.45 | 95.33 | 148.25 |
|  | $3+$ | 114.96 | 96.14 | 89.88 | 121.22 |
| Protein | 1 | 173.21 | 188.64 | $212.71{ }^{\text {a }}$ | $149.14{ }^{\text {b }}$ |
|  | 2 | 124.64 | 102.42 | 126.00 | 101.06 |
|  | $3+$ | 102.03 | 107.50 | 106.50 | 103.03 |
| $\text { Persistency }{ }^{3}$ |  |  |  |  |  |
| Milk | 1 | 0.02 | -0.19 | -0.10 | -0.07 |
|  | 2 | -0.51 | -0.89 | $-0.16^{\text {a }}$ | $-1.24{ }^{\text {b }}$ |
|  | $3+$ | -0.50 | -0.97 | $-0.22^{\text {a }}$ | $-1.25{ }^{\text {b }}$ |
| Fat | 1 | $0.00{ }^{\text {b }}$ | $0.02{ }^{\text {a }}$ | 0.02 | 0.00 |
|  | 2 | -0.02 | -0.04 | $-0.01{ }^{\text {a }}$ | $-0.05{ }^{\text {b }}$ |
|  | $3+$ | -0.02 | -0.05 | $-0.02{ }^{\text {a }}$ | $-0.05{ }^{\text {b }}$ |
| Protein | 1 | $0.01{ }^{\text {b }}$ | $0.03{ }^{\text {a }}$ | 0.02 | 0.01 |
|  | 2 | -0.01 | -0.02 | $0.00{ }^{\text {a }}$ | $-0.02{ }^{\text {b }}$ |
|  | $3+$ | -0.01 | -0.02 | $0.00{ }^{\text {a }}$ | $-0.02{ }^{\text {b }}$ |

Table 3.2 Continued

| Curve Trait | Parity | Feeding system |  | Animal Breed |  |
| :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | Traditional | TMR | Brown <br> Swiss | Holstein <br> Friesian |
| Mean |  |  |  |  |  |
| Milk | 1 | $2.20{ }^{\text {b }}$ | $4.60{ }^{\text {a }}$ | $2.14{ }^{\text {b }}$ | $4.66{ }^{\text {a }}$ |
|  | 2 | $2.55{ }^{\text {b }}$ | $5.42{ }^{\text {a }}$ | $2.53{ }^{\text {b }}$ | $5.45{ }^{\text {a }}$ |
|  | 3+ | $2.58{ }^{\text {b }}$ | $5.44{ }^{\text {a }}$ | $2.57{ }^{\text {b }}$ | $5.45{ }^{\text {a }}$ |
| Fat | 1 | $0.07{ }^{\text {b }}$ | $0.19{ }^{\text {a }}$ | 0.11 | 0.14 |
|  | 2 | $0.08{ }^{\text {b }}$ | $0.23{ }^{\text {a }}$ | 0.14 | 0.17 |
|  | $3+$ | $0.08{ }^{\text {b }}$ | $0.23{ }^{\text {a }}$ | 0.14 | 0.17 |
| Protein | 1 | $0.07{ }^{\text {b }}$ | $0.17{ }^{\text {a }}$ | 0.09 | 0.15 |
|  | 2 | $0.08{ }^{\text {b }}$ | $0.19{ }^{\text {a }}$ | 0.11 | 0.17 |
|  | 3+ | $0.08{ }^{\text {b }}$ | $0.19^{\text {a }}$ | 0.10 | 0.16 |

[^0]Descriptive statistics for each chemical parameter of TMR samples collected from the 28 farms involved in the project are shown in Table 3.3. Correlations among chemical components are shown in Table 3.4. A negative correlation between DM and SN was observed. This was possibly because of wet forages (silages) in the TMR, which often have higher SN content than dry forages. However, some farms also added water to their TMR, which also may have reduced DM and increased SN. The significantly high ( $\mathrm{P}=0.001$ ) positive correlation between SN and ADF suggested the possible use of wet citrus products. Positive correlations among ADL, ADF, and NDF, and negative correlations between starch and ADL, ADF, and NDF were consistent: higher ADL content means higher content of both NDF and ADF, but lower content of starch.

Table 3.3 Mean chemical composition of TMR samples collected from 28 farms in Ragusa province.

|  | Mean | SD | Minimum | Maximum |
| :--- | ---: | ---: | ---: | ---: |
| Dry matter (\%) | 93.40 | 1.24 | 90.74 | 95.30 |
| Ash (\% of DM) | 7.83 | 0.69 | 6.67 | 9.54 |
| Crude protein (\% of DM) | 15.44 | 1.78 | 11.95 | 24.40 |
| Soluble nitrogen (\% of DM) | 32.43 | 5.58 | 21.64 | 48.63 |
| Acid detergent lignin (\% of DM) | 4.13 | 0.78 | 2.39 | 6.46 |
| Neutral detergent fiber (\% of DM) | 40.47 | 4.00 | 25.97 | 48.60 |
| Acid detergent fiber (\% of DM) | 22.57 | 2.39 | 17.00 | 29.97 |
| Starch (\% of DM) | 20.93 | 2.88 | 14.18 | 28.46 |

Table 3.4 Pearson's correlations among chemical properties of TMR samples collected from 28 farms in Ragusa province.

| Item | Ash | Soluble N | ADL | ADF |  | NDF |  | CP |  | Starch |
| :--- | ---: | ---: | ---: | ---: | :--- | :--- | :--- | :--- | :--- | :--- |
| Dry matter | 0.19 | $-0.29^{*}$ | 0.10 | -0.35 | $* *$ | -0.01 |  | 0.07 | 0.11 |  |
| Ash | 1 | $-0.34^{*}$ | 0.21 | 0.25 |  | 0.25 | -0.23 | -0.31 | $*$ |  |
| Soluble Nitrogen | 1 | 0.18 | 0.45 | $* * *$ | 0.19 | 0.15 | -0.35 | $* *$ |  |  |
| Acid Detergent Lignir |  | 1 | 0.39 | $* *$ | 0.52 | $* * *$ | -0.15 | -0.32 | $*$ |  |
| Acid Detergent Fiber |  |  | 1 |  | 0.53 | $* * *$ | -0.31 | $*$ | -0.63 | $* * *$ |
| Acid Detergent Fiber |  |  |  |  | 1 | -0.39 | $* * *$ | -0.40 | $* * *$ |  |
| Crude Protein |  |  |  |  |  | 1 | 0.03 |  |  |  |

Results of multiple regression analysis performed to estimate association of average TMR chemical composition with HCUR traits are shown in Table 3.5. The CP content of the TMR had the greatest effect of composition of TMR for all effects (Table 3.5).

Table 3.5 Values of $R^{2}$ and estimates (SE) for the regression parameters after using multiregression with backward and forward elimination ${ }^{1}$

|  | Parity | $\mathrm{R}^{2}$ | DM |  | CP |  | DM $\times$ CP |  | NDF×starch |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Peak |  |  |  |  |  |  |  |  |  |
| Milk | 1 | 0.24 |  |  | 0.277 | *** |  |  |  |
|  | 2 | 0.18 |  |  | 0.242 | ** |  |  |  |
|  | $3+$ | 0.14 |  |  |  |  | 0.002 | ** |  |
| Fat | 1 | 0.14 |  |  |  |  | 0.002 | ** |  |
|  | 2 | 0.22 |  |  | 0.270 | *** |  |  |  |
|  | $3+$ | 0.15 |  |  | 0.232 | ** |  |  |  |
| Protein | 1 | 0.17 |  |  | 0.243 | ** |  |  |  |
|  | 2 | 0.14 |  |  | 0.218 | ** |  |  |  |
|  | 3+ | 0.12 |  |  |  |  | 0.002 | * |  |
| DIM at Peak |  |  |  |  |  |  |  |  |  |
| Milk | 1 | 0.2 |  |  |  |  | -0.002 | * |  |
|  | 2 | 0.13 |  |  |  |  |  |  | $0.003^{* *}$ |
|  | $3+$ | n.s. |  |  |  |  |  |  |  |
| Fat | 1 | 0.27 |  |  |  |  |  |  |  |
|  | 2 | 0.12 |  |  | -0.201 | ** |  |  |  |
|  | $3+$ | 0.22 |  |  | -0.164 | * |  |  |  |
| Protein | 1 | 0.14 | -0.236 | * |  |  |  |  |  |
|  | 2 | n.s. |  |  |  |  |  |  |  |
|  | $3+$ | 0.06 |  |  |  |  |  |  |  |
| Persistency ${ }^{4}$ |  |  |  |  |  |  |  |  |  |
| Milk | 1 | 0.18 |  |  | 1.526 | * | -0.019 | ** |  |
|  | 2 | 0.33 |  |  |  |  | -0.003 | *** | 0.002 |
|  | 3+ | 0.30 |  |  |  |  | -0.003 | *** | 0.002 |
| Fat | 1 | 0.14 | -0.005 | * |  |  |  |  |  |
|  | 2 | 0.39 |  |  | 1.133 | * | -0.015 | * |  |
|  | 3+ | 0.29 |  |  |  |  | -0.003 | *** |  |
| Protein | 1 | 0.06 | -0.226 | * |  |  |  |  |  |
|  | 2 | 0.32 |  |  | 1.575 | ** | -0.019 | ** | 0.002 |
|  | 3+ | 0.27 |  |  | 1.312 | * | -0.016 | * | 0.002 |

Table 3.5 Contiued.

| Mean |  |  |  |  |
| :--- | ---: | ---: | :--- | :--- |
| Milk | 1 | 0.18 | 0.246 | $* *$ |
|  | 2 | 0.14 | 0.220 | $* *$ |
|  | $3+$ | 0.14 | 0.216 | $* *$ |
| Fat | 1 | 0.16 | 0.241 | $* * *$ |
|  | 2 | 0.13 | 0.217 | $* *$ |
|  | $3+$ | 0.12 | 0.211 | $* *$ |
| Protein | 1 | 0.16 | 0.228 | $* *$ |
|  | 2 | 0.12 | 0.204 | $* *$ |
|  | $3+$ | 0.11 | 0.196 | $* *$ |

${ }^{1}$ The regressions are in SD units for y per unit x , where $\mathrm{y}=$ herd curve traits for milk, fat, and protein and $\mathrm{x}=$ average TMR chemical properties. Ash, soluble nitrogen, acid detergent lignin, NDF, ADF, and all their interactions with all other variables were included in the set of regressors, but no significant effect was found.
${ }^{2}$ persistency was estimated as $P=\frac{1}{245} \sum_{i=61}^{305} y_{i}-y_{60}$ (Kistemaker, 2003), where $y_{i}=$ yield at DIM $i$.

* $\mathrm{P}<0.05 ;{ }^{* *} \mathrm{P}<0.01 ;{ }^{* * *} \mathrm{P}<0.001$.

Crude protein had a significant effect ( $\mathrm{P}<0.05$ ) on peak and mean HCUR for all traits and for all parities. An interaction between CP and DM was significantly associated with persistency for milk HCUR for all parities, but for fat and protein HCUR for parities 2 and 3+ only. A significant effect was found for DM on fat and protein HCUR persistency for parity 1. Mean values for each curve trait were estimated for farms using a TMR with all the extreme values of CP (13.25 $\pm 0.76$ $16.01 \pm 0.45$ ) and DM ( $91.13 \pm 0.37-94.49 \pm 0.56$ ). Figures 3.1 and 3.2 show average $(\mathrm{n}=3)$ HCUR of second-parity protein and fat yields for the combination of extreme values of CP and DM; low CP-high DM farms had the lowest peak and mean values for milk production HCUR (1.8 and 1.6 respectively) compared with high CP-high DM farms (9.9 and 8.6, respectively).


Figure 3.1 Average ( $n=3$ ) herd curve of second-parity protein yield for combination of extreme values of CP and DM (•LL = low content of both CP and DM, $\bullet L H=$ low content of CP and high content of DM, $\mathbf{\Delta H L}=$ high content of $C P$ and low content of DM, and $\mathbf{~ H H}=$ high content of both CP and DM).


Figure 3.2 Average ( $n=3$ ) herd curve of second-parity fat yield for combination of extreme values of CP and DM (•LL = low content of both CP and DM, •LH = low content of CP and high content of DM, $\mathbf{\Delta H L}=$ high content of $C P$ and low content of DM, and $\mathbf{- H H}=$ high content of both CP and DM).

### 3.4 DISCUSSION

## Animal breed

Previous research has reported differences in milk and milk component yields due to breed (McDowell and McDaniel, 1968; Brandt et al., 1974; Dechow et al., 2007; Walsh et al., 2008). In this study, the major differences were found between Holstein Friesian and Brown Swiss farms in HCUR peak for milk yield and persistency for milk, fat, and protein yields. In general, herd curves of Holstein Friesian farms had higher milk peak but were less persistent for all traits compared with Brown Swiss farms. Holstein Friesian cows have been chosen by farmers for their greater milk productivity compared with other breeds. However, to some extent the observation was surprising because breed effect is already accounted for by the pedigree structure in the TD model and assigning unknown parents to phantom groups that differed by breed. The effect observed here suggests that there are more effects with breed than can be explained by the genetic makeup of the individual animals alone. These are likely management effects that are confounded with breed and were picked up in the random herd curves analyzed in this study. Another reason could be that breed effect is adjusted at the TD level and not as a random effect on overall herd production. One solution to adjust for these effects in the RRTDM would be to include a random curve for breed. This would remove breed differences, allowing advisors to give management advice independent of breed. Walsh et al. (2008) explored the influence of breed and feeding system on milk production, body weight, body condition score, reproductive performance, and postpartum ovarian function. Holstein Friesian animals produced the greatest yield of solids-corrected milk. As observed by Walsh et al. (2008), differences observed between the different breeds were a likely consequence of the selection criteria adopted for each breed. Similarly, Mc-Dowell and McDaniel (1968), Brandt et al. (1974), and Dechow et al. (2007) found that pure Holstein Friesian had the highest milk yield production compared with Brown Swiss.

Milk HCUR persistencies presented in this research are consistent with those of McDowell and McDaniel (1968). However, Brandt et al. (1974) found higher persistency for milk produced by Holstein Friesian cows compared with Brown Swiss. Although the definition of persistency was the same as in the McDowell and McDaniel work, the difference in results could be due to the selection of animals in the experiment.

## Feeding systems

Significant differences ( $\mathrm{P}<0.001$ ) for peak and mean HCUR for all production traits were found between feeding systems (SF vs. TMR) in this study. The TMR-fed cows produced, on average, more milk, fat, and protein, and their curves had a higher peak compared with animals fed with SF. The effect was consistent for all parities in all traits except for milk in parities 2 and $3+$. Several studies have compared TMR feeding systems (often referred to as complete feed or complete diet) with SF systems, where the forage and concentrate components of the diet are offered to cows separately.
Bargo et al. (2002) compared 3 feeding systems combining pasture and TMR (pasture plus concentrate, pasture plus partial TMR, and TMR) and found that the TMR feeding system resulted in the highest total dry matter intake and milk production: cows on the TMR treatment produced $6.1 \mathrm{~kg} / \mathrm{d}$ more milk compared with cows on a partial TMR treatment. Gordon et al. (1995) found that feeding a complete diet resulted in $3.04 \mathrm{~kg} / \mathrm{d}$ more milk than feeding concentrate separately from silage without altering milk concentrations of fat and protein. In our study, herds fed the TMR had $1.88,2.17$, and $2.17 \mathrm{~kg} / \mathrm{d}$ greater HCUR mean for parities 1 , 2, and 3+, respectively. Similarly for milk composition, Bargo et al. (2002) found that the use of TMR increased milk fat percentage and true protein ( 0.35 and 0.34 more, respectively, than partial TMR). In our study we found that TMR herds produced more mean HCUR fat ( $0.10,0.13$, and $0.12 \mathrm{~g} / \mathrm{d}$, for parities 1,2 , and $3+$, respectively) and protein ( $0.07,0.09$, and $0.09 \mathrm{~g} / \mathrm{d}$, for parities 1,2 , and $3+$ respectively).
On the contrary, several studies have reported different results. In Gordon et al. (1995), a review of 13 comparisons of TMR versus SF showed that in most of the studies, feeding system had no or only a small effect on milk composition. Ferris et al. (2006) conducted 2 experiments to examine performance of dairy cows associated with 2 winter feeding systems (daily complete diet feeding vs. separate feeding of the forage and concentrate components). Feeding system had no significant effect on any aspect of performance of the dairy cows measured or on nutrient utilization. Animal performance was measured as total milk output throughout the experiments, milk per day, and milk composition ( $\mathrm{g} / \mathrm{kg}$ of milk). Bargo et al. (2002) concluded that milk yield responses to TMR were most likely to occur when studies involved high-yielding cows ( $>28 \mathrm{~kg}$ of milk per day) in early lactation. Yrjänen et al. (2003) found that feeding concentrate with 2 different strategies (SF vs. TMR) had no effect ( $P>0.05$ ) on milk production and milk composition. However, differences over the lactation curve were found: cows fed with SF produced more milk in early lactation, whereas cows fed TMR produced
more milk later in the lactation period. As suggested by Yrjänen et al. (2003), the lack of difference in milk production and better response to SF in early lactation could be explained by the fact that cows were fed using a computerized self-feeder that allowed them to consume moderate levels of concentrate as frequent meals during the day. In classical SF strategies, feeding concentrates 2 or 3 times a day can have detrimental effects on rumen environment because the amount of concentrate is a major factor influencing rumen pH . All of these studies are based on experiments where individual animals were fed somewhat constant nutritive components, differing only in the strategies used to feed the animals. In the current study, herds using different feeding strategies were selected randomly to assess whether feeding management represents one source of variation in herd mean milk rather than individual cow milk responses.

## Nutritional Composition

In this study, a significant effect ( $\mathrm{P}<0.05$ ) of CP on peak and mean HCUR for all production traits was found. The interaction CP $\times$ DM had a significant effect ( $\mathrm{P}<$ 0.01 ) on persistency for all traits and parities, except for fat and protein for firstparity cows, whereas NDF $\times$ starch marginally affected $(P<0.1)$ persistency for milk and protein HCUR in parities 2 and 3 . These results confirmed those from other studies. Wu and Satter (2000) investigated milk production response in highproducing dairy cows to dietary supplementation with different amounts of protein. Cows fed diets with greater CP content (18\%) achieved greater peak production, but had a decrease in milk production later in lactation almost identical to that in cows fed lower protein, suggesting that the highest protein did not affect the latter part of the lactation. Law et al. (2009) found that an increase in dietary CP concentration significantly increased milk, fat, and protein yields in early lactation (d 1 to 150), arguing that thereafter, protein concentration can be reduced with no detrimental effects on animal performance. Holter et al. (1997) found high correlations of dietary CP with milk and milk protein yield ( $r=42$ and $38 \%$, respectively). However, Broderick (2003) reported that increasing dietary protein concentration above $167 \mathrm{~g} / \mathrm{kg}$ of DM had only small positive effects on dry matter intake and milk and protein yields. Similarly, Cunningham et al. (1996) found that increasing the amount of CP in diets had only small effects on the pattern of amino acids in duodenal digesta. Consequently, when diets contained higher amounts of CP, the yields of milk and milk components improved, probably because of higher flows of nitrogen and essential amino acids to the intestine. In the same study, when dry matter intake of cows was higher, there appeared to be little advantage in increasing the percentage of dietary CP , underscoring how dry
matter intake can affect the response of lactating dairy cows to dietary concentrations of CP. Hristov et al. (2002) performed a meta-analysis based on nutritional studies published in the Journal of Dairy Science (volumes 73 through 82) to determine dietary factors affecting milk yield and milk protein yield in dairy cows. Correlations between milk yield and milk protein yield and dietary composition variables were poor.
Based on the meta-analysis performed by Hristov et al. (2002), higher effects of energy (starch) and forage quality (ADL, ADF, and NDF) were expected on herd curve traits. One reason could be that the association between yearly average diet composition on the average herd curve for production was investigated. The average chemical composition may have reduced the variability between TMR within herd, some of the explored effects, in particular energy and forage quality, might become more evident when combining the nutrition information with HCUR at the corresponding stage of lactation (beginning, peak, or end). For example, the TMR fed prior or during peak lactation might affect the shape of the curve more than the TMR fed on average across the lactation. This requires further investigations. It is also necessary to assess whether energy in the diet affects milk production at the cow level by examining individual animal curves or deviations of real from expected production estimated from the model.

### 3.4 CONCLUSION

Results from this analysis demonstrated that CP and DM content in the diet and their interaction significantly influence HCUR traits, especially peak, mean, and persistency. Herd curves therefore are useful to warn farmers about inappropriate feeding. However, this advice cannot be given without correcting properly for breed and feeding system, which are shown to be important sources of variation in herd milk and milk component yield curves. When feeding a TMR it is important to pay particular attention to DM and CP content in the diet. As a tool for farm managers, HCUR can be considered a good indicator of herd management related to feeding management by examining abnormal shapes or negative values of HCUR traits.

## Acknowledgments

The authors thank people from CoRFiLaC (Ragusa, Italy) for their support: in particular, Giuseppe Azzaro, Emiliano Gurrieri, Mariano Gambina, and Carmelo Scollo at the Extension Group for data collection, and Salvatore Ventura for his help
in HCUR generation. Financial support was provided by the Assessorato Agricoltura e Foreste della Regione Siciliana (Palermo, Italy).

## REFERENCES

AOAC. 1994. Official Methods of Analysis. 15th ed. AOAC, Arlington, VA.
AOAC. 1998. Official Methods of Analysis. 4th rev. 16th ed. AOAC International, Arlington, VA.
Bargo, F., L. D. Muller, J. E. Delahoy, and T. W. Cassidy. 2002. Performance of highproducing dairy cows with three different feeding systems combining pasture and total mixed rations. J. Dairy Sci. 85:2948-2963.
Brandt, G. W., C. C. Brannon, and W. E. Johnston. 1974. Production of milk and milk constituents by Brown Swiss, Holsteins, and their crossbreds. J. Dairy Sci. 57:1388-1393.

Broderick, G. A. 2003. Effects of varying dietary protein and energy levels on the production of lactating dairy cows. J. Dairy Sci. 86:1370-1381.

Caccamo, M., R. F. Veerkamp, G. de Jong, M. H. Pool, R. Petriglieri, and G. Licitra. 2008. Variance components for test-day milk, fat and protein yield, and somatic cell scores for analyzing management information. J. Dairy Sci. 91:3268-3276.
Coppock, C. E. 1977. Feeding methods and grouping systems. J. Dairy Sci. 60:13271336.

Cunningham, K. D., M. J. Cecava, R. Johnson, and P. A. Ludden. 1996. Influence of source and amount of dietary protein on milk yield by cows in early lactation. J. Dairy Sci. 79:620-630.
Dechow, C. D., G. W. Rogers, J. B. Cooper, M. I. Phelps, and A. L. Mosholder. 2007. Milk, fat, protein, somatic cell score, and days open among Holstein, Brown Swiss, and their crosses. J. Dairy Sci. 90:3542-3549.
De Roos, A. P. W., A. G. F. Harbers, and G. de Jong. 2004. Random herd curves in a test-day model for milk, fat, and protein production of dairy cattle in the Netherlands. J. Dairy Sci. 87:2693-2701.
Everett, R. W., F. Schmidt, and L. H. Wadell. 1994. A test-day model for monitoring management and genetics in dairy cattle. J. Dairy Sci. 77(Suppl. 1):267 (Abstr.).

Ferris, C. P., J. P. Frost, R. C. Binnie, and D. C. Patterson. 2006. Dairy cow performance and labour inputs associated with two silage feeding systems. Grass Forage Sci. 61:304-314.
Goering, H. K., and P. J. Van Soest. 1970. Forage fiber analysis (apparatus, reagent, procedures and some applications). Agriculture Handbook No. 379. US Department of Agriculture, Washington, DC.

Gordon, F. J., D. C. Patterson, T. Yan, M. G. Porter, C. S. Mayne, and E. F. Unsworth. 1995. The influence of genetic index for milk production on the response to complete diet feeding and the utilization of energy and nitrogen. Anim. Sci. 61:199-210.

Holter, J. B., J. W. West, and M. L. McGilliard. 1997. Predicting ad libitum dry matter intake and yield of Holstein cows. J. Dairy Sci. 80:2188-2199.

Hristov, A. N., W. J. Price, and B. Shafii. 2002. Dietary factors influencing milk yield and milk protein yield in dairy cows. Pages 147-166 in Proc. Pacific Northwest Animal Nutr. Conf., Vancouver, BC, Canada. C R Press, Portland, OR.
Kirkpatrick, M., D. Lofsvold, and M. Bulmer. 1990. Analysis of the inheritance, selection and evolution of growth trajectories. Genetics 124:979-993.
Kistemaker, G. J. 2003. Comparison of persistency definitions in random regression test day models. Interbull Bull. 30.
Law, R. A., F. J. Young, D. C. Patterson, D. J. Kilpatrick, A. R. G. Wylie, and C. S. Mayne. 2009. Effect of dietary protein content on animal production and blood metabolites of dairy cows during lactation. J. Dairy Sci. 92:1001-1012.
Licitra, G., T. M. Hernandez, and P. J. Van Soest. 1996. Standardization of procedures for nitrogen fractionation of ruminant feeds. Anim. Feed Sci. Technol. 57:347-358.

Mayeres, P., J. Stoll, J. Bormann, R. Reents, and N. Gengler. 2004. Prediction of dailymilk, fat, and protein production by a random regression test-day model. J. Dairy Sci. 87:1925-1933.

McDowell, R. E., and B. T. McDaniel. 1968. Interbreed matings in dairy cattle. I. Yield traits, feed efficiency, type and rate of milking. J. Dairy Sci. 51:767-777.

NRS. 2009. E7; Breeding value estimation of milk production traits with test-day model. http://www.nrs.nl/index-eng.htm Accessed May 2010.

Pool, M. H., and T. H. E. Meuwissen. 1999. Prediction of daily milk yields from a limited number of test days using test day models. J. Dairy Sci. 82:1555-1564.
Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583-3597.

Walsh, S., F. Buckley, K. Pierce, N. Byrne, J. Patton, and P. Dillon. 2008. Effects of breed and feeding system on milk production, body weight, body condition score, reproductive performance, and postpartum ovarian function. J. Dairy Sci. 91:4401-4413.
Wu, Z., and L. D. Satter. 2000. Milk production during the complete lactation of dairy cows fed diets containing different amounts of protein. J. Dairy Sci. 83:1042-1051.

Yrjänen, S., K. Kaustell, R. Kangasniemi, J. Sariola, and H. Khalili. 2003. Effects of concentrate feeding strategy on the performance of dairy cows housed in a free stall barn. Livest. Prod. Sci. 81:173-181.

## 4

## Association of total-mixed-ration chemical composition with milk, fat, and protein yield lactation curves at the individual level

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#### Abstract

The objective of this study was to examine the effect of the chemical composition of a total mixed ration (TMR) tested quarterly from March 2006 through December 2008 for milk, fat, and protein yield curves for 27 herds in Ragusa, Sicily. Before this study, standard yield curves were generated on data from 241,153 testday records of 9,809 animals from 42 herds in Ragusa province collected from 1995 to 2008. A random regression sire-maternal grandsire model was used to develop variance components for yields. The model included parity, age at calving, year at calving, and stage of pregnancy as fixed effects. Random effects were herd $\times$ test date, sire and maternal grandsire additive genetic effect, and permanent environmental effect modeled using third-order Legendre polynomials. Model fitting was carried out using ASREML. Subsequently, the model with estimated variance components was used to examine the influence of TMR crude protein, soluble nitrogen, acid detergent lignin, neutral detergent fiber, acid detergent fiber, starch, and ash on milk, fat, and protein yield curves. The data set contained 46,531 test-day milk yield records from 3,554 cows in the 27 herds recorded during the study period. Initially, an analysis was performed using one dietary component (one-component analysis) within each model as a fixed effect associated with the test-day record closest to the months the TMR was sampled within each herd. An interaction was included with the nutrient component and days in milk. The effect of the TMR chemical component(s) was modeled using a ninth-order Legendre polynomial. The conditional Wald F-statistic for the fixed effects revealed significant effects ( $\mathrm{P}<$ 0.001 ) for acid detergent fiber, neutral detergent fiber, crude protein, starch, and their interactions with days in milk on milk, fat, and protein yield. On the basis of these results, a multicomponent analysis was performed in which crude protein, neutral detergent fiber, and starch were simultaneously included in the model with days in milk interactions. Although both analyses revealed that diet composition influenced production responses depending on lactation stage, the multiplecomponent analysis showed more pronounced effects of starch and neutral detergent fiber relative to crude protein for all traits throughout lactation.


Key words: lactation curve, total mixed ration, testday model

### 4.1 INTRODUCTION

Lactation curves for milk and milk components in dairy cattle show variation in peak and persistency of yield, partially explained by dietary composition and feeding management. It has long been recognized that the increase in milk production decreases for each unit increase in crude protein (CP) with increased CP content of the diet (Wu et al., 2000, Ipharraguerre and Clark, 2005). Source of dietary protein influences rumen degradability and has a modifying effect on production responses at moderate (around 16\% CP) dietary CP concentrations (Reynal and Broderick, 2003, Ipharraguerre and Clark, 2005). Furthermore, starch and total energy content of the diet may modify responses to CP. Cabrita et al. (2007) observed that low-protein, low-starch diets decreased dry matter intake and milk production in mid-lactation cows, but milk production responded to increases in dietary CP, starch, or both. Hristov et al. (2002) observed that starch (energy content) and forage quality significantly affected herd curve traits, whereas Oba and Allen (2003) observed that cows in early lactation fed high-starch diets (32\% of dry matter) versus low-starch diets ( $21 \%$ of dry matter) produced more milk and protein. In addition, dry corn versus high-moisture corn was associated with higher fat content and fat production in cows fed the high-starch diet.
High-starch diets typically have lower fiber content; therefore, it is difficult to separate the effects of increased starch content from the effects of lower dietary fiber. Weiss et al. (2009) observed that the major response in milk volume and milk components was to changes in metabolizable CP supply and not to starch when starch varied from 22.0 to $30.0 \%$ of DM. Brun-Lafleur et al. (2010) varied energy and CP content across 9 diets fed to lactating dairy cows and observed that production responses to increasing dietary energy were dependent on adequate CP supply. Production responses to dietary content were greater for cows with high milk production potential, and first-lactation cows responded differently from multiparous cows. This means that the milk production response to changing the diet is dependent on the production potential of the cows, the stage of lactation, management, and the relative concentrations of dietary nutrients. Therefore, a better understanding of the effects of diet on production requires a methodology that controls for lactation stage, management, and cow factors across multiple diets.
Test-day (TD) models provide insight into variation in lactation curves for individual cows and herds and have been used to suggest management advice (Koivula et al., 2007; Caccamo et al., 2008; Halasa et al., 2009). Caccamo et al. (2008) observed that the variation between herd lactation curves for milk and protein yields was
highest around the time of peak yield. Subsequently, in an analysis using the TD model to examine factors influencing production at the herd level, Caccamo et al. (2010) observed that animal breed (Holstein-Friesian vs. Brown Swiss) affected peak, persistency, and mean lactation curves for all production traits. Furthermore, the feeding system [total mixed ration (TMR) vs. separate feeding] influenced peak and mean milk production for all traits and parities. When looking at the average nutrient composition of TMR fed over the year, only CP and dry matter content in the diet and their interaction significantly influenced herd curve traits, peak, persistency, and mean milk yield. To be able to extrapolate dietary factors to management advice for individual cows, it is necessary for the diet composition to be linked with individual cow lactation curves. To do this at the field level requires that the TD records be associated with the period that the known diet composition was fed.

A possible reason that associations for other nutrient variables were not observed in the previous study by Caccamo et al. (2010) was that yearly average diet composition was assessed against yearly average herd curves for production. Using the average chemical composition of sampled TMR across the year may have reduced the variability between TMR within the herd over the course of a year and masked some of the effects of nutrient composition, in particular starch and forage quality. It was thought that an investigation of dietary content aligned more closely to TD production might reveal more sensitive relationships between dietary nutrients and milk production. The objective of this study was, therefore, to assess the association of the nutrient composition of the TMR with cow lactation curves for milk, fat, and protein yield and to compare the estimates of a single-component analysis with a model that takes into account all dietary components simultaneously. Using deviations from predictions of production from the TD model for individual cows would control for the effects of herd, parity, stage of lactation, season, and genetics and enable a more sensitive analysis of nutrient effects on production across days in milk.

### 4.2 MATERIALS AND METHODS

## Data

Production data for milk (kg), fat (g), and protein (g) and TMR information were collected from 27 herds located in Ragusa province (Italy) from 2006 through 2008, forming a data set that included 46,531 TD records from 3,554 cows. This data set was used to estimate the association of random individual curves for milk yield
with chemical composition of the diets. To estimate variance components for the genetic effects more precisely, a larger data set (full data set) with more animals than the ones with known TMR was necessary. A full data set that included 241,153 TD records from 9,809 animals in 42 herds recorded from 1995 through 2008 was supplied by the local milk recording agency (APA Ragusa, Ragusa, Italy) and used to estimate variance components for milk (kg), fat (g), and protein (g) yield by using a random regression TD model.

For the 27 herds included in the reduced data set, TMR samples were collected every 3 mo from March 2006 through December 2008 and analyzed for ash (AOAC, 1994), crude protein (CP, AOAC, 1994), soluble nitrogen (Licitra et al., 1996), acid detergent lignin (ADL, Goering and Van Soest, 1970), neutral detergent fiber (NDF, Van Soest et al., 1991), acid detergent fiber (ADF, Goering and Van Soest, 1970), and starch (AOAC, 1998; method 996.11). All chemical analyses were expressed on a dry matter basis.
Diets were also evaluated using CPM Dairy (version 3.0.8; University of Pennsylvania, Kennett Square, PA, Cornell University, Ithaca, NY, and Miner Agricultural Research Institute, Chazy, NY).

## Estimation of Variance Components

Production TD records for the full data set was processed using a multiplelactation, single-trait random regression TD model:

$$
\begin{aligned}
& \mathrm{y}_{\mathrm{dklmnnoprs}}=\mathrm{ay}_{\mathrm{k}}+\mathrm{ppr}_{\mathrm{l}}+\mathrm{pdd} \mathrm{~m}_{\mathrm{m}}+\mathrm{ym}_{\mathrm{n}}+\mathrm{htd} \mathrm{o}_{\mathrm{o}}+ \\
& +\sum_{\mathrm{q}=0}^{2} \mathrm{z}_{\mathrm{q}}\left(\mathrm{as}_{\mathrm{qs}}+1 / 2 \mathrm{ams}_{\mathrm{qs}}\right)+\sum_{\mathrm{q}=0}^{2} \mathrm{z}_{\mathrm{q}}\left(\mathrm{pe}_{\mathrm{qs}}\right)+\mathrm{e}_{\mathrm{dklmnoprs}}
\end{aligned}
$$

where $\mathrm{y}_{\mathrm{dk} / \mathrm{mnoprs}}$ is the yield record (milk, fat, or protein yield) of cow s on days in milk (DIM) $d$ of parity $p$ in herd $r$ within herd test date effect $n$ and belonging to fixed effect class $k, I, m$, and $n$ defined as follows: $a y_{k}$ is the kth class of age at calving $\times$ year of calving ( 23 classes); ppr, is lth class of the parity $\times$ stage of pregnancy ( 135 classes); $\operatorname{pdd}_{m}$ is the $m$ th class of parity $\times$ days dry ( 153 classes); $\mathrm{ym}_{\mathrm{n}}$ is the nth class of year of test $\times$ month of test ( 55 classes); htd ${ }_{\mathrm{o}}$ is the random herd $\times$ test date o ( 1,386 classes); $z_{q}$ is the $q$ order Legendre polynomial (Kirkpatrick et al., 1990); as $\mathrm{q}_{\mathrm{qs}}$ is the random additive genetic effect of sire of cow s corresponding to polynomial q ; $\mathrm{ams}_{\mathrm{qs}}$ is the random additive genetic effect of the maternal grandsire of cow s corresponding to polynomial q ; $\mathrm{pe}_{\mathrm{qs}}$ is the random permanent environmental effect of cow $s$ corresponding to polynomial $q$; and $\mathrm{e}_{\mathrm{dk} \text { lmnoprs }}$ is the residual belonging to observation $\mathrm{y}_{\mathrm{djk} \text { knoprs }}$.

The pedigree file included 178 sires and 305 grandsires. Unknown parents were assigned to 5 phantom pedigree groups based on their breed (Holstein-Friesian, Brown Swiss, Simmenthal, Modicana, crossbreed, and unknown). Random effects were herd test date, sire and maternal grandsire additive genetic effect, and permanent environmental effect modeled using third-order Legendre polynomials. This order was chosen based on the fit: higher order models gave converging problems and did not explain more variance. Model fitting was carried out using ASREML (Gilmour et al., 2009).

## Association between TMR Chemical Composition and Lactation Curve

First, a one-component analysis for diet nutrition components (NutUni) was run, in which the variables describing TMR chemical composition were included in the above model one by one. Because TD records were collected monthly whereas TMR were sampled every 3 mo, each TD record was associated with the closest TMR analysis fed to animals immediately before or after the TD. The effect of TMR chemical components was modeled as an interaction with DIM using a ninth-order Legendre polynomial to increase the sensitivity to dietary effects across DIM. Based on the results of the NutUni analysis, a multiple-component analysis (NutMulti) was performed, in which CP, NDF, and starch and their interaction with DIM (fitted as a ninth-order Legendre polynomial) were simultaneously included in the model The significance of effects was tested using the conditional Wald F-statistic in the NutUni and NutMulti models (Gilmour et al., 2009).

Values of parameters estimated in the NutUni and NutMulti models were used to generate lactation curves for milk, fat, and protein yield for the average nutrient value(s) as well as plus and minus 2 standard deviations of nutrient values of the TMR. For the prediction models, average values for fixed effects were held constant, random effects were ignored, and curves were generated for each given value(s) for TMR chemical content, varied as above, obtaining marginal prediction curves between 5 and 305 DIM (Gilmour et al., 2004). In the NutMulti analysis, prediction curves did not include the effects of interactions between chemical nutrients and DIM.

### 4.3 RESULTS

Descriptive statistics of the chemical composition of TMR samples collected from the 27 farms involved in the project are shown in Table 4.1, whereas pairwise Pearson correlations among them are shown in Table 4.2. Mean content of TMR (SD) as a percentage of dry matter for CP, NDF, ash, and starch were 15.5 (1.96), 40.4 (4.51), 8.0 (1.14), and 20.3 (3.72; Table 4.1). Positive correlations ( $P<0.001$ )
among ADL, ADF, and NDF, and negative correlations between starch and ADL, ADF, and NDF were as expected: higher ADL content was associated with higher content of both NDF and ADF but lower content of starch. In addition, increasing the content of NDF was associated with a decreasing content of starch ( $r=-0.44$ ) and CP ( $r=-0.29$ ) in TMR in this study (Table 4.2). In general, higher fiber content meant less energy and CP in the diet.

Table 4.1 Mean composition of chemical properties of TMR samples collected from 27 farms in Ragusa province.

| Item (\% of DM) | Mean | Range <br> (minimum-maximum) | SD |
| :--- | :---: | :---: | :---: |
| Ash | 8.0 | $5.9-15.9$ | 1.14 |
| Crude protein | 15.5 | $11.1-32.4$ | 1.96 |
| Soluble nitrogen | 31.9 | $11.2-63.7$ | 7.06 |
| Acid detergent lignin | 4.2 | $1.0-11.9$ | 1.25 |
| Acid detergent fiber | 23.1 | $13.7-32.1$ | 3.35 |
| Neutral detergent fiber | 40.4 | $24.2-54.5$ | 4.51 |
| Starch | 20.3 | $7.6-32.3$ | 3.72 |

Table 4.2 Pearson correlations among chemical properties of TMR samples collected from 27 farms in Ragusa province.

| Item (\% of DM) | CP | Soluble N | ADL | ADF |  | NDF |  | Starch |  |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Ash | -0.06 | 0.05 | 0.11 | 0.20 | $* *$ | 0.05 |  | -0.25 | $* * *$ |
| Crude protein | - | 0.02 | -0.09 | -0.29 | $* * *$ | -0.35 | $* * *$ | 0.16 | $*$ |
| Soluble nitrogen | - | 0.10 | 0.32 | $* * *$ | 0.14 | $*$ | -0.34 | $* * *$ |  |
| Acid detergent ligin |  | - | 0.49 | $* * *$ | 0.35 | $* * *$ | -0.26 | $* * *$ |  |
| Acid detergent fiber |  |  | - |  | 0.53 | $* * *$ | -0.66 | $* * *$ |  |
| Neutral detergent fiber |  |  |  |  | - |  | -0.44 | $* * *$ |  |
| Starch |  |  |  |  |  |  | - |  |  |

$$
\text { *P < 0.05; **P < 0.01; ***P < } 0.001 .
$$

In the NutUni analysis, all chemical parameter values, except ash, and their interactions with DIM influenced ( $\mathrm{P}<0.001$ ) milk lactation curves (Table 4.3). The main factors influencing fat and protein ( $\mathrm{P}<0.01$ ) were ash, ADF, NDF, CP, and starch (Table 4.3). Dietary nutrients were both negatively and positively correlated
with each other, as expected. Production effects in NutUni for individual nutrients may be confounded by correlated changes with other dietary concentrations of nutrients. For example, an increase in ash content of the TMR was associated with a reduction in starch content and an increase in ADF content of the TMR. Therefore, production responses in the NutUni models may not be very revealing. Other than CP, increases in dietary content of ash, ADF, NDF, and ADL were all associated with significant decreases in starch content of the TMR (Table 4.2). Therefore, to more closely examine production influences, multiple-component models were also examined for dietary nutrients.
Lignin and ADF are subcomponents of NDF; NDF represents the main structural carbohydrate in dairy rations. Starch is the major non-structural carbohydrate in dairy rations, often comprising 60 to $75 \%$ of the non-fiber carbohydrate fraction, with the remainder composed of sugars, soluble fibers, and silage acids. Protein forms the other major fermentable organic component of dairy rations. Therefore, NDF, starch, and CP were selected for inclusion in the multiple-component analysis of dietary components on milk volume and content.

Predicted production curves for milk and fat yields (kg/d) for TMR content of CP (Figures 4.1 and 4.2, respectively), starch (Figures 4.3 and 4.4, respectively), and NDF (Figures 4.5 and 4.6, respectively) are presented in Figures 4.1 through 4.6 Two sets of curves are plotted within each figure. Production curves with dashed lines represent the NutUni predictions for the effect of the main nutrient by DIM with no other nutrients in the model; the production curves with solid lines present the NutMulti predictions based on inclusion of CP, starch, and NDF. Production curves were generated based on mean nutrient content of TMR (mean: 15.5\% CP 20.3\% starch, 40.4\% NDF), 2 standard deviations below the mean concentration of TMR (low: 11.6\% CP, 12.8\% starch, 31.4\% NDF), and 2 standard deviations above the mean concentration of TMR (high: $19.4 \%$ CP, $27.7 \%$ starch, $49.5 \%$ NDF). In the NutUni prediction models (dashed curves), no other nutrients were included in the model; therefore, the curves represent the singular effects of changing the dietary content of the nutrient of interest. The production curves predicted from the NutMulti model (solid lines) present the effects of changing the concentration of the nutrient of interest with the dietary concentration of the other 2 nutrients in the model set to mean, high, or low. The change in production curves with other nutrients included in the model (NutMulti) suggests the confounding response one can have when multiple nutrients are not accounted for in the NutUni models. Protein yield curves are not presented because they closely followed the milk volume curves.
4 - Diet chemical composition effect on individual lactation curves
Table 4.3 Probability values for the Wald statistic of the association between TMR chemical components and their interaction with DIM with milk, fat, and protein yield ${ }^{1}$.

| Item | Milk |  | Fat |  | Protein |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | NutUni | NutMulti | NutUni | NutMulti | NutUni | NutMulti |
| Ash | 0.328 | $-^{2}$ | < . 001 | - | 0.002 | - |
| Ash x DIM | $<.001$ | - | 0.003 | - | < . 001 | - |
| Soluble N | < .001 | - | 0.709 | - | 0.858 | - |
| Soluble $\mathrm{N} \times$ DIM | < . 001 | - | 0.292 | - | 0.352 | - |
| ADL ${ }^{2}$ | < . 001 | - | 0.723 | - | 0.002 | - |
| ADL x DIM | <. 001 | - | 0.038 | - | 0.086 | - |
| ADF | <. 001 | - | < . 001 | - | < . 001 | - |
| ADF x DIM | < . 001 | - | < . 001 | - | 0.005 | - |
| NDF | < . 001 | $<.001$ | < . 001 | 0.152 | < . 001 | $<.001$ |
| NDF x DIM | <. 001 | < . 001 | 0.149 | 0.014 | 0.002 | < . 001 |
| CP | <. 001 | <. 001 | < . 001 | < . 001 | <. 001 | <. 001 |
| CP x DIM | < . 001 | <. 001 | <. 001 | 0.002 | <. 001 | 0.002 |
| Starch | <. 001 | < . 001 | < . 001 | < . 001 | < . 001 | < . 001 |
| Starch x DIM | $<.001$ | <. 001 | <. 001 | < . 001 | < . 001 | $<.001$ |

Estimations were performed using a one-component analysis (NutUni) and a multiple-component analysis (NutMulti) for diet nutrient components.
${ }^{2}$ The term was not included in the analysis.


Figure 4.1 Lactation curves estimated using the NutUni (dashed lines; onecomponent analysis) and the NutMulti (solid lines; multiple-component-analysis) model for milk (kg), predicted at different contents (minimum curve = average - 2 SD, light grey lines; average curve, dark grey lines; maximum curve = average +2 SD, black lines) of CP in TMR at 3 different combinations with starch and NDF.


Figure 4.2 Lactation curves estimated using the NutUni (dashed lines; onecomponent analysis) and the NutMulti (solid lines; multiple-component-analysis) model for fat (g), predicted at different contents (minimum curve = average -2 SD, light grey lines; average curve, dark grey lines; maximum curve = average +2 SD, black lines) of CP in TMR at 3 different combinations with starch and NDF.


Figure 4.3 Lactation curves estimated using the NutUni (dashed lines; onecomponent analysis) and the NutMulti (solid lines; multiple-component-analysis) model for milk (kg), predicted at different contents (minimum curve = average - 2 SD, light grey lines; average curve, dark grey lines; maximum curve = average +2 SD, black lines) of starch in TMR at 3 different combinations with CP and NDF.


Figure 4.4 Lactation curves estimated using the NutUni (dashed lines; onecomponent analysis) and the NutMulti (solid lines; multiple-component-analysis) model for fat (g), predicted at different contents (minimum curve = average -2 SD, light grey lines; average curve, dark grey lines; maximum curve = average +2 SD, black lines) of starch in TMR at 3 different combinations with CP and NDF.


Figure 4.5 Lactation curves estimated using the NutUni (dashed lines; onecomponent analysis) and the NutMulti (solid lines; multiple-component-analysis) model for milk ( kg ), predicted at different contents (minimum curve $=$ average -2 SD, light grey lines; average curve, dark grey lines; maximum curve = average +2 SD, black lines) of NDF in TMR at 3 different combinations with CP and starch.


Figure 4.6 Lactation curves estimated using the NutUni (dashed lines; onecomponent analysis) and the NutMulti (solid lines; multiple-component-analysis) model for fat (g), predicted at different contents (minimum curve = average -2 SD, light grey lines; average curve, dark grey lines; maximum curve = average +2 SD, black lines) of NDF in TMR at 3 different combinations with CP and starch.

Figure 4.1 shows milk production responses by DIM for changes in CP content (Figure 4.1). As expected CP, increased peak milk production ( $\mathrm{P}<0.001$ ) in both the NutUni and NutMulti analyses (Table 4.3, Figure 4.1). Further, increasing dietary CP increased milk production in late lactation but had little effect in mid-lactation in the NutUni curves (Figure 4.1). However, the effect of changing dietary CP on milk production across all DIM was influenced by dietary starch and NDF content. With the minimal starch ( $12.8 \%$ of DM) and maximal NDF concentration ( $49.5 \%$ of DM), little production response to changing the CP concentration was observed (Figure 4.1c). Only small differences were observed in mid-lactation, and increased CP was associated with slightly lower production. With mean starch ( $20.3 \%$ of DM) and NDF ( $40.4 \%$ of DM) concentrations in TMR, increasing the CP content of diets increased peak milk yield, but little production change was observed after 125 DIM until small increases were apparent after 215 DIM (Figure 4.1a). With average starch and NDF content, increasing CP from 11.6\% of TMR to $15.5 \%$ increased peak milk yield by approximately $0.5 \mathrm{~kg} / \mathrm{d}$ (Figure 4.1a). When starch was $27.7 \%$ and NDF was $31.4 \%$ of DM in the TMR, increasing CP from 11.6 to $15.5 \%$ increased peak milk yield by approximately $0.9 \mathrm{~kg} / \mathrm{d}$ (Figure 4.1b). When CP was increased to $19.4 \%$ of TMR, with mean starch and NDF, peak milk yield increased by an additional 0.6 $\mathrm{kg} / \mathrm{d}$, whereas with the greater starch and lower NDF dietary concentrations, peak yield increased by an additional $0.9 \mathrm{~kg} / \mathrm{d}$ from the mean CP concentration. In addition, CP increased production throughout lactation when starch was high and NDF was low. When starch content was $27.7 \%$ and NDF was $31.4 \%$, increasing dietary CP increased peak milk yield and yield throughout lactation. For the optimal production response to CP , starch must be high and NDF low.
Figure 4.2 shows the yield of fat ( $\mathrm{kg} / \mathrm{d}$ ) for changing dietary CP. Increasing CP content of the TMR in the NutUni prediction increased peak fat yield (fewer than 120 DIM), and then after 200 DIM to 305 DIM (Figure 4.2, dashed lines). Very early in lactation, at fewer than 20 DIM, increasing dietary CP was associated with lower fat yields (Figure 4.2, dashed line curves). In the NutMulti model, increasing TMR CP increased fat yield throughout lactation; however, responses were greatest at peak milk yield and after 200 DIM for all starch and NDF concentrations. However, the yield increases in milk fat were greatest for high-starch and low-NDF TMR content, with responses to increasing CP being intermediate with mean starch and mean NDF content in the TMR (Figure 4.2, solid lines in panels a, b, and c). An inverse effect of CP content on fat yield in the first 20 d of lactation was also apparent in the NutMulti models. This maybe an artifact because few TD records are collected from cows at fewer than 20 DIM; however, mobilization of tissue reserves may also confound responses to dietary nutrients within this time period.

Increasing dietary starch always increased milk yield throughout lactation DIM in NutUni and NutMulti (Figure 4.3). When CP was $11.6 \%$ and NDF was 49.5\%, increasing dietary starch from 11.6 to $20.3 \%$ of DM increased milk yield by approximately $0.4 \mathrm{~kg} / \mathrm{d}$. Further increasing starch to $27.7 \%$ of dry matter when CP was low and NDF was high increased milk yield an additional $0.4 \mathrm{~kg} / \mathrm{d}$. When CP and NDF were at the mean dietary concentrations, milk yield increased by $1.4 \mathrm{~kg} / \mathrm{d}$, almost 3 times the response when CP was low and NDF was high, when starch was increased from a low to mean concentration in the TMR. Further increasing starch to $27.7 \%$ of DM increased milk yield by an additional $1.4 \mathrm{~kg} / \mathrm{d}$. When CP was high and NDF was low, increasing dietary starch to $20.3 \%$ of dry matter from a low concentration increased milk yield by $2.2 \mathrm{~kg} / \mathrm{d}$ (Figure 4.3b). Increasing starch to a high concentration increased milk yield by an additional $2.2 \mathrm{~kg} / \mathrm{d}$. Therefore, the response to starch was enhanced by increased dietary CP and decreased dietary NDF concentration in the TMR.
In the NutUni model, increasing dietary starch influenced milk fat yield in very early lactation (Figure 4.4, dashed lines). The high concentration of dietary starch was associated with low fat yields in the first month of lactation (Figure 4.4, dashed lines). Low dietary starch was associated with greater fat yields in early lactation in the NutUni model. However, these associations were removed in the NutMulti model. Increasing dietary starch had little influence on milk fat yield with low CP and high NDF in the TMR (Figure 4.4c, solid lines). When CP and NDF were included in the model at mean concentrations, the response in milk fat yield to increasing starch content was approximately 30 g when starch content increased from low (12.8\%) to mean (20.3\%) and from mean to high (27.7\%; Figure 4a), and peak fat yield was approximately 30 DIM. When CP was high and NDF was low, the response to increasing starch content from low (12.8\%) to mean (20.3\%) TMR concentration was approximately 60 g of fat yield throughout lactation (Figure 4.4b). Further increasing starch to high concentration (27.7\%) increased fat yield by an additional $60 \mathrm{~g} / \mathrm{d}$ throughout lactation (Figure 4.4b, solid lines). Peak fat yield to increasing starch was approximately 50 DIM when CP was high and NDF was low (Figure 4.4b). Increasing starch increased fat yield when CP and NDF were at least mean to high, and low, respectively, in TMR.
In the NutUni model for NDF, low NDF was associated with greater milk yields than mean and high-NDF TMR concentrations (Figure 4.5, dashed lines). The effect of changing NDF content in the TMR was more apparent when CP and starch were high in TMR (Figure 4.5b). The response to decreasing NDF was approximately 1 $\mathrm{kg} / \mathrm{d}$ when reducing the concentration from 49.5 to $40.4 \%$ when CP and starch were high in TMR (Figure 4.5b), whereas the milk response was approximately 0.5
$\mathrm{kg} / \mathrm{d}$ when CP and starch were mean in TMR (Figure 4.5a), and the milk response to decreasing NDF content was less apparent when CP and starch were low in TMR (Figure 4.5c). Milk yield increased by an additional $1 \mathrm{~kg} / \mathrm{d}$ when NDF was reduced from 40.4 to $31.4 \%$ when CP and starch were high in the TMR, whereas the response was approximately $0.5 \mathrm{~kg} / \mathrm{d}$ when the TMR had mean CP and NDF concentrations (Figure 4.5a).
Low NDF content increased fat yield by approximately $9 \mathrm{~g} / \mathrm{d}$ compared with mean NDF TMR content and by approximately $18 \mathrm{~g} / \mathrm{d}$ compared with high-NDF TMR content throughout lactation in the NutUni model (Figure 4.6). In the NutMulti model, the influence of changing TMR NDF content on fat yield was reversed when responses for low TMR content of CP and starch were compared with high TMR content of CP and starch (Figure 4.6, panels a, b, and c). When starch and CP were low, decreasing the NDF from high to mean content increased fat yield by $30 \mathrm{~g} / \mathrm{d}$ throughout lactation (Figure 4.6c), and when NDF decreased from mean to low content, fat yield increased by an additional $30 \mathrm{~g} / \mathrm{d}$ (Figure 4.6 c ). The response to altered NDF when starch and CP were at mean concentrations in the TMR was interesting. Fat yields for the 3 NDF concentrations were almost superimposed (Figure 4.6a), and yield had a peak at 20 to 30 d postcalving. When starch and CP TMR concentrations were high, high NDF was associated with the greatest fat yield (Figure 6b) and low NDF content was associated with the lowest fat yield relative to the mean and high-NDF content (Figure 4.6b).

Dietary starch was the major dietary factor influencing all traits ( $\mathrm{P}<0.001$ ) throughout lactation in both the NutUni and NutMulti analyses (Table 4.3). Increasing starch had a significant direct effect on milk production throughout lactation. However, when also accounting for CP and NDF in the NutMulti analysis, the effect of starch became much more pronounced when CP was high and NDF was low. Taken together, increasing starch content of the TMR had approximately twice the influence on milk volume compared with increasing CP (compare Figure 4.1, panels $a$ and $b$, with Figure 4.3, panels $a$ and $b$ ). The more pronounced effect of starch on fat yield is also apparent when comparing Figure 4.2, panels $a$ and $b$, with Figure 4.4, panels $a$ and $b$. The effect of dietary CP on milk yield and fat yield is fully expressed when starch is high and NDF is low.

### 4.4 DISCUSSION

The effects of nutritional composition on lactation curves for milk, fat, and protein yield have been investigated extensively in experiments with varying nutritional quality (Wu and Satter, 2000; Ipharraguerre et al., 2005; Brun-Lafleur et al., 2010).

In this study, we used a different kind of approach, in which we retrospectively explained the variation in the lactation curves attributable to diet composition. The disadvantages of our approach was that the TMR composition was examined only 4 times a year for nutritional components, and these were associated with the closest TD milk yield. However, the advantage was that a large number of herds and animals were included in an analysis that controlled for genetic and herd-yearseason effects. We were also able to investigate many nutritional components simultaneously by quantifying the substitution effect on production (i.e., the change in production attributable to increasing or decreasing one diet component when the other components were fixed at 3 dietary concentrations) under the assumption that animals fed free choice eat to their maximal capacity. Several experiments would have been needed to investigate these interactions between components.

## Nutritional Effects of Single Components

Results of responses in this study suggest that changes in milk yield attributable to CP and NDF were dependent on dietary content of starch. When starch was high and NDF was low, the response to increasing CP content of the TMR was most prominent. Likewise, when starch and CP were high, the milk response to decreasing dietary NDF was greatest. Overall, starch content of the TMR was the most important element in determining increased milk and fat yields. Increasing starch always increased milk and fat yields. Dietary CP was the second most important factor. However, the response to CP was strongly dependent on starch and NDF content of the TMR. Effects of CP on yield were dependent on mean to high starch and mean and low NDF content of the TMR. Reducing the TMR NDF content increased the yield of milk, but the effects were most pronounced when starch and CP were high. Fat yields were increased by dietary starch, particularly when CP and NDF concentrations were mean concentrations or CP was high and NDF was low in the TMR. Likewise, increasing CP increased fat yield, but not to the extent of starch, when NDF was mean or low and CP was mean or high. When starch and CP were low in TMR, then decreasing NDF content increased fat yields, but when CP and starch were high, then increasing NDF content increased fat yield. Despite the difference in methodology, the effects of nutritional components on lactation curves found in this study were consistent with the reported literature, which lends credibility to the multiple-component models. Increasing CP content increased peak milk yield and had small effects in late lactation. Responses to changing concentrations of CP in the single-nutrient model and the multiplenutrient model under average conditions were similar. Wu and Satter (2000)
observed that increasing dietary CP from 15 to 17 to $18 \%$ increased peak milk yield, and $17 \%$ CP throughout lactation was recommended for maximizing total yield. However, they found that after 14 wk of lactation, CP could be reduced to $16 \%$ of the diet with only small reductions in yield and significant reductions in total nitrogen excretion. The milk curves in this paper suggest dietary CP influences peak milk production, and dietary concentration of CP in mid-lactation was not very important until after 250 DIM, and even then effects on milk production were small. Law et al. (2009) found that an increase in dietary CP concentration increased milk, fat, and protein yield in early lactation (d 1 to 150), arguing that thereafter, CP concentration can be reduced with no detrimental effects on animal performance. Holter et al. (1997) found high correlations of dietary CP with milk and milk protein yield ( $r=42$ and $38 \%$, respectively). However, Broderick (2003) reported that increasing dietary CP concentration above $167 \mathrm{~g} / \mathrm{kg}$ of DM had only small positive effects on dry matter intake, with no increases in milk, fat, or protein yield. Similarly, Cunningham et al. (1996) found that increasing the amount of CP in diets had only small effects on the pattern of amino acids in duodenal digesta because of variable effects of rumen degradation of dietary CP. Consequently, when diets contained amounts of CP above $16 \%$ of DM, compared with a diet of $14.5 \%$ CP, yields of milk and milk components improved. However, increasing dietary CP to $18 \%$ of dry matter did not improve milk or milk component yields. Milk production improved when dietary CP supplied higher flows of nitrogen and essential amino acids to the intestine.

Flow of nonammonia nitrogen and essential amino acids to the small intestine is a function of dry matter intake, CP content of the ration, rumen degradation of feed protein, and the flow of microbial protein from the rumen. Ipharraguerre et al. (2005) observed that cows consuming adequate energy and other nutrients received sufficient amino acids flow to the small intestine when consuming 14 to $18 \%$ CP diets and observed no production response in cows consuming adequate energy. Therefore, CP effects on milk production may be apparent only at peak milk production, when energy intake is limiting, and may not be significantly apparent in latter lactation, when energy intake is not limiting. Furthermore, because NDF and starch content of the diet influence microbial growth, the influence of dietary CP on small intestinal amino acids flow is dependent on these other dietary constituents. In this study, dietary CP of $19.4 \%$ was associated with higher peak milk yield compared with 15.5 and $11.6 \%$ dietary CP. However, it appeared advantageous to feed this amount of CP only in early lactation, and responses to CP were dependent on increasing starch and reducing NDF.

Starch content of the TMR became the most significant factor influencing milk, fat, and protein yield over the entire lactation. Increasing starch content of the TMR elevated the entire lactation curve and increased fat yield throughout lactation. Protein yield followed a pattern similar to fat yield (data not shown). Therefore, starch content of the TMR is an important factor influencing milk volume and fat and protein yields. Starch influences not only potential energy supply but also metabolizable CP supply by association with rumen microbial synthesis.
Feeding more than $20 \%$ starch throughout lactation would be recommended, based on the results of this study. Caution should be exercised in interpreting $27.7 \%$ as the optimum starch content to feed because only 3 dietary concentrations of starch were explored in this study (12.8, 20.3, and 27.7\%): between 20 and $27 \%$ starch, contents that are intermediate and more economically optimal may be available, although these were not explored in this paper.

## Interactions between Components

It is impossible to alter one nutrient concentration because values need to sum to $100 \%$ of the diet. We chose to examine CP, starch, and NDF because these are the more common nutrients nutritionists are attempting to control in dairy rations. Responses to high concentrations of NDF, CP, and starch should be interpreted cautiously because these values sum to $96.7 \%$ of dry matter, which is unlikely to be observed in many herd situations. Mean ash was $8.0 \%$ and mean fat content was $4.4 \%$ of the TMR in this study (data not shown). The low end of the range of ash was $5.9 \%$, and fat was $2.2 \%$ (data not shown); therefore, the high TMR content for CP, NDF, and starch could not exist together. Given the correlations in Table 4.2, as NDF increases, starch and CP in the TMR would decrease (approximately at equal magnitudes), whereas CP and starch (more weakly) would tend to change in concert with each other (Table 4.2). Therefore, increases in NDF should be focused on curves with reducing CP and starch content in the TMR.
Although increasing starch had a significant effect on milk production throughout lactation in both analyses, a more pronounced effect for starch was found in the NutMulti model than in the NutUni model. Brun-Lafleur et al. (2010) found similar results when assessing the effect of the energy $\times$ protein interaction on milk yield: this interaction resulted in a sharper response of milk yield to energy supply for high levels than for low levels of CP supply. When CP is at an average concentration in the diet, increasing starch had a slightly larger effect on increasing the peak yield than did increasing CP when starch was at an average concentration. This could be due to an effect of balancing of amino acids, glucose, and acetate: when CP content is increased at the expense of starch, some of the amino acids can be used for
glucose, but not vice versa. On a nutrient efficiency basis, increasing starch in moderate-CP diets to increase peak milk yield would be a more attractive strategy than increasing CP, and less nitrogen would be wasted in urinary excretion. Reynolds et al. (2001) infused increasing amounts of starch into the duodenum and observed decreases in urinary nitrogen output along with increases in milk production and body tissue deposition. As lactation proceeds, increasing CP would not benefit production unless starch is high. For example, at approximately 150 DIM, if starch and NDF are mean, milk yield is slightly greater than 32 kg of milk/d for all CP concentrations (Figure 4.1a). However if starch is high and NDF is low, then milk yield is slightly greater than $36 \mathrm{~kg} / \mathrm{d}$ for high CP concentration, slightly lower than $36 \mathrm{~kg} / \mathrm{d}$ for mean CP concentration, and approximately $35 \mathrm{~kg} / \mathrm{d}$ for low CP concentration. Therefore, milk yield increased more by high starch and low NDF than by changing CP (Figure 4.1b). Contrary to this study, Broderick (2003) found no energy $\times$ protein interaction effect on milk production. Similarly, Cabrita et al. (2007) observed that increasing CP from approximately $13.5 \%$ to approximately $15.5 \%$ when starch was $15 \%$ of dry matter resulted in production responses similar to increasing starch to $23.9 \%$ of dry matter when CP was $13.5 \%$. These studies were designed to examine starch $\times$ CP interactions, whereas the present study is based on an a priori decision to examine milk curves for diets fed to animals in commercial herds.
Starch nutrient content of $27.7 \%$ appeared to have the greatest effect on milk, fat, and protein yield. Starch has the dual effect not only of supplying energy, but also of being a significant source of protein for the cow because of rumen utilization of starch for microbial CP synthesis. As long as sufficient nitrogen is present in the rumen, fermentation of starch can provide significant amounts of microbial CP. The NRC (2001) uses a conversion of 130 g of bacterial $\mathrm{CP} / \mathrm{kg}$ of total digestible nutrients. Increasing starch from 20.3 to $27.7 \%$ would potentially increase bacterial CP supply by 45 to $60 \mathrm{~g} / \mathrm{d}$ if NDF remains $40 \%$ of dry matter. A confounding factor in actual fact is that when starch is increased, some other dietary components must change because these are expressed as a percentage of dry matter, which has to add to $100 \%$. However, in the responses in this paper, increasing starch to $27.7 \%$ should be accompanied with a reduction in NDF to 31.4 from $40.4 \%$. This change is a trade-off of a more rumen fermentable carbohydrate (starch) for a less rumen degradable carbohydrate (NDF). In this study, if NDF is $31.4 \%$ of dry matter in the TMR, CP is $18.5 \%$, and starch is $24 \%$, if CP is increased to $19.5 \%$, milk would increase by approximately $0.2 \mathrm{~kg} / \mathrm{d}$. However, if starch were increased to $25 \%$ with CP at $18.5 \%$, milk would increase by $0.5 \mathrm{~kg} / \mathrm{d}$ (combining responses in milk curves from Figures 4.1 and 4.3). Fat yield would change very little with the increase in CP
in this scenario, but increasing starch would increase fat yield by approximately 8 $\mathrm{g} / \mathrm{d}$ (combining Figures 4.2 and 4.4). A unit increase in dietary starch content has a greater effect on milk and fat yield than does a unit increase in CP. Reduction in NDF typically means a reduction in forage in the ration, for which a limit exists regarding how much forage can be reduced and still maintain rumination and a rumen mat of long particles. A minimum NDF concentration of $31.4 \%$ should be adequate because the NRC (2001) recommends a minimum of 25 to $33 \%$ dietary NDF.

The change in production effect when the other nutrients are included in the model found in this study suggests the confounding response one can have when multiple nutrients are not accounted for. The integration of information from the NutMulti curves for CP, starch, and NDF suggests that diets should contain moderate CP, high starch, and low NDF content in the first 150 DIM to maximize milk volume and fat and protein yields. In this study, those nutrient concentrations would be $19.4 \%$ CP, $27.7 \%$ starch, and $31.4 \%$ NDF. After 150 DIM, production was always higher with the $27.7 \%$ starch diet. Fat and protein yields were not influenced by CP content in late lactation, and milk volume was influenced only to a modest extent. Combined, after 150 DIM it appears that diets with $27.7 \%$ starch, $40.4 \%$ NDF, and 11.5 to $15.5 \%$ CP content would maintain yields of milk, fat, and protein. In any case, this study has developed a statistical model able to describe variation in milk and milk component yields when the diet composition of the TMR changes. Results from extreme conditions have to be carefully interpreted because only 3 dietary conditions for each component were examined: intermediate values (not explored in this study) would be more appropriate in terms of feed costs and production response while preventing metabolic disorders.

### 4.4 CONCLUSION

Using data collected at farms in a field study with a modeling approach, effects of CP, NDF, and starch on individual lactation curves for milk, fat, and protein could be reproduced. Fitting the interaction between the diet components showed that starch had the greatest effect on milk, fat, and protein production when CP and NDF contents were at a high and low value, respectively. To accomplish feeding the appropriate ration based on DIM and production, farms should group cows accordingly to minimize under- and overfeeding.

## Acknowledgments

The authors thank Giuseppe Giurdanella for data editing. Financial support was provided by the Assessorato Agricoltura e Foreste della Regione Siciliana (Palermo, Italy).

## REFERENCES

AOAC. 1994. Official Methods of Analysis. 15th ed. Assoc. Off. Anal. Chem., Arlington, VA.
AOAC. 1998. Official Methods of Analysis of AOAC International. $4^{\text {th }}$ rev. 16 th ed. Assoc. Off. Anal. Chem. Int., Arlington, VA.
Broderick, G. A. 2003. Effects of varying dietary protein and energy levels on the production of lactating dairy cows. J. Dairy Sci. 86:1370-1381.
Brun-Lafleur, L., L. Delaby, F. Husson, and P. Faverdin. 2010. Predicting energy $\times$ protein interaction on milk yield and milk composition in dairy cows. J. Dairy Sci. 93:4128-4143.

Cabrita, A. R. J., R. J. B. Bessa, S. P. Alves, R. J. Dewhurst, and A. J. M. Fonseca. 2007. Effects of dietary protein and starch on intake, milk production, and milk fatty acid profiles of dairy cows fed corn silage-based diets. J. Dairy Sci. 90:1429-1439.
Caccamo, M., R. F. Veerkamp, G. de Jong, M. H. Pool, R. Petriglieri, and G. Licitra. 2008. Variance components for test-day milk, fat and protein yield, and somatic cell scores for analyzing management information. J. Dairy Sci. 91:3268-3276.
Caccamo, M., R. F. Veerkamp, J. D. Ferguson, R. Petriglieri, F. La Terra, and G. Licitra. 2010. Associations of breed and feeding management with milk production curves at herd level using a random regression test-day model. J. Dairy Sci. 93:4986-4995.
Cunningham, K. D., M. J. Cecava, R. Johnson, and P. A. Ludden. 1996. Influence of source and amount of dietary protein on milk yield by cows in early lactation. J. Dairy Sci. 79:620-630.
Gilmour, A., B. Cullis, S. Welham, B. Gogel, and R. Thompson. 2004. An efficient computing strategy for prediction in mixed linear models. Comput. Stat. Data Anal. 44:571-586.
Gilmour, A. R., B. J. Gogel, B. R. Cullis, and R. Thompson. 2009. ASReml User Guide. NSW Dept. Primary Industries, Orange, Australia.
Goering, H. K., and P. J. Van Soest. 1970. Forage fiber analysis (apparatus, reagent, procedures and some applications). Agric. Handbook No. 379. US Dept. Agric., Washington, DC.

Halasa, T., M. Nielen, A. P. W. De Roos, R. Van Hoorne, G. de Jong, T. J. G. M. Lam, T. van Werven, and H. Hogeveen. 2009. Production loss due to new subclinical
mastitis in Dutch dairy cows estimated with a test-day model. J. Dairy Sci. 92:599-606.
Holter, J. B., J. W. West, and M. L. McGilliard. 1997. Predicting ad libitum dry matter intake and yield of Holstein cows. J. Dairy Sci. 80:2188-2199.
Hristov, A. N., W. J. Price, and B. Shafii. 2002. Dietary factors influencing milk yield and milk protein yield in dairy cows. Pages 147-166 in Proc. Pacific Northwest Animal Nutr. Conf., Vancouver, British Columbia, Canada. C R Press, Portland, O.
Ipharraguerre, I. R., J. H. Clark, and D. E. Freeman. 2005. Varying protein and starch in the diet of dairy cows. I. Effects on ruminal fermentation and intestinal supply of nutrients. J. Dairy Sci. 88:2537-2555.
Kirkpatrick, M., D. Lofsvold, and M. Bulmer. 1990. Analysis of the inheritance, selection and evolution of growth trajectories. Genetics 124:979-993.
Koivula, M., J. I. Nousiainen, J. Nousiainen, and E. A. Mäntysaari. 2007. Use of herd solutions from a random regression test-day model for diagnostic dairy herd management. J. Dairy Sci. 90:2563-2568.
Law, R. A., F. J. Young, D. C. Patterson, D. J. Kilpatrick, A. R. G. Wylie, and C. S. Mayne. 2009. Effect of dietary protein content on animal production and blood metabolites of dairy cows during lactation. J. Dairy Sci. 92:1001-1012.
Licitra, G., T. M. Hernandez, and P. J. Van Soest. 1996. Standardization of procedures for nitrogen fractionation of ruminant feeds. Anim. Feed Sci. Technol. 57:347-358.
NRC. 2001. Nutrient Requirements of Dairy Cattle. 7th Rev. Ed. National Research Council. Nat. Acad. Sci., Washington, DC.
Oba, M., and M. S. Allen. 2003. Effects of corn grain conservation method on feeding behavior and productivity of lactating dairy cows at two dietary starch concentrations. J. Dairy Sci. 86:174-183.
Reynal, S. M., and G. A. Broderick. 2003. Effects of feeding dairy cows protein supplements of varying ruminal degradability. J. Dairy Sci. 86:835-843.
Reynolds, C.K., S.B. Cammell, D.J. Humphries, D.E. Beever, J.D. Sutton, J.R. Newbold. 2001. Effects of postrumen starch infusion on milk production and energy metabolism in dairy cows. J. Dairy Sci. 84:2250-2259.
Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583-3597.
Weiss, W. P., N. R. St-pierre, and L. B. Willett. 2009. Varying type of forage, concentration of metabolizable protein, and source of carbohydrate affects nutrient digestibility and production by dairy cows. J. Dairy Sci. 92:5595-5606.

Wu, Z., and L. D. Satter. 2000. Milk production during the complete lactation of dairy cows fed diets containing different amounts of protein. J. Dairy Sci. 83:1042-1051.

## 5

## Association of total-mixed-ration particle fractions retained on the Penn State Particle Separator with milk, fat, and protein yield lactation curves at individual level

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To be submitted to Journal of Dairy Science


#### Abstract

As part of a larger project aiming to develop management evaluation tools based on results from test-day (TD) models, the objective of this study was to examine the effect of physical composition of total mixed ration (TMR) tested quarterly from March, 2006 through December, 2008 on milk, fat, and protein yield curves for 25 herds in Ragusa, Sicily. A random regression sire - maternal grandsire model was used to estimate variance components for milk, fat, and protein yields fitted on a full dataset including 241,153 TD records from 9,809 animals in 42 herds recorded from 1995 through 2008. The model included parity, age at calving, year at calving, and stage of pregnancy as fixed effects. Random effects were herd $\times$ test date, sire and maternal grandsire additive genetic effect, and permanent environmental effect modeled using third-order Legendre polynomials. Model fitting was carried out using ASREML. Afterwards, for the 25 herds involved in the study, 9 particle size classes were defined based on the proportions of TMR particles on the top and middle screen of the Penn State Particle Separator (PSPS). Subsequently, the model with estimated variance components was used to examine the influence of TMR particle size class on milk, fat, and protein yield curves. An interaction was included with the particle size class and DIM. The effect of the TMR particle size class was modeled using a ninth-order Legendre polynomial. Lactation curves were predicted from the model while controlling for TMR chemical composition (crude protein content of $15.5 \%$, neutral detergent fiber content of $40.7 \%$ and starch of $19.7 \%$ for all classes), in order to have purely estimates of particle distribution not confounded by nutrient content of TMR. Little effect of class of particle proportions on milk yield and fat yield curves was found. Surprisingly, protein yield was significantly greater for sieve classes with 10.4 to $17.4 \%$ of TMR particles retained on the upper PSPS 19 mm sieve. Optimal distributions different than those recommended may reflect regional differences based on types of forages fed.


Key words: lactation curve, particle size, TMR, test day model

### 5.1 INTRODUCTION

Adequate fiber [plant residues insoluble in neutral detergent after Van Soest et al. (1991), NDF] is important in dairy rations to support normal rumen activity and prevent milk fat depression and health problems associated with rumen acidosis. However, NDF alone is an insufficient measure of adequacy of fiber in dairy rations, as it does not account for the physical form. Rations may contain adequate NDF, but be processed so finely that normal rumen activity cannot be maintained. Mertens (1997) combined the concept of adequate chemical NDF with physical form to define physically effective NDF (peNDF) as a measure that captures the physical characteristics of fiber by accounting for particle length and NDF content. Physically effective NDF promotes chewing and the flow of salivary buffers to the rumen to maintain a normal rumen milieu (Mertens, 1997). As mean particle size decreases, chewing time and rumen pH decline due to a reduction in saliva production and its buffering action (Woodford and Murphy, 1988; Grant and Colenbrander, 1990a, 1990b).
Physically effective fiber is the fraction of the diet that stimulates chewing (NRC, 2001). To ensure adequate fiber, the NRC (2001) recommends that diets comprised primarily of corn silage and alfalfa haylage as forage sources and dry corn as the main concentrate source contain a minimum of $25 \%$ NDF on a dry matter (DM) basis and $76 \%$ of the NDF should be from forage NDF. The dietary concentration of NDF can be increased based on alterations in amounts of forage in the ration and particle size of the ration, but standard guidelines are qualitative in nature. Typical values for NDF in corn silage and alfalfa haylage may range from 38 to $46 \%$ of DM and 36 to $45 \%$ of DM, respectively. Thus forage content of diets may vary from $43 \%$ to $58 \%$ of total DM consumed to meet these guidelines. However, these guidelines don't specify a particle size, which can vary greatly depending on the chop length set at harvest and mixing of forages within a TMR, which may ultimately influence the effectiveness of the NDF to maintain a rumen mat and adequate chewing activity.
A challenge has been establishing a method to define peNDF in dairy rations. Lammers et al. (1996) developed a simple field usable device to estimate particle size of forages and TMR (Penn State Particle Separator, PSPS). The PSPS was designed to allow separation of feed particles by a shaking motion duplicating vertical sieving. Initially two screens, 19.0 mm and 8.0 mm , and a pan were used to estimate mean particle size. Since that publication, the PSPS has been modified to include a third screen, 1.18 mm in size (Kononoff et al. 2003a). Guidelines published by Penn State Extension recommends that adequate chewing is
maintained when a TMR contains 2 to $8 \%$ of material on the top screen ( 19.0 mm ), 30 to $50 \%$ of material on the middle screen ( 8 mm ), 30 to $50 \%$ of material on the lower screen ( 1.18 mm ) and $<20 \%$ of material in the pan. Most authors have focused on the total feed material retained on the top two screens as physically effective material in dairy rations. In fact Schadt et al. (2012) found that masticates from hay particles retained on the top two screens contained particles sufficiently long enough to contribute to long particles in the rumen. Particles retained on the 1.18 mm screen when masticated were too fine to contribute to formation of the rumen mat.
Various estimates have been used to estimate peNDF from particle distribution in the PSPS. The simplest is based on as fed distribution of feed particles, with the proportion retained on the top two screens as an estimate of the peNDF. Further refinements include DM retained on the top two screens times the NDF content of the entire diet, NDF retained on the top two screens as a proportion of total NDF, and other modifications. In addition, mean geometric particle size has been calculated based on proportions retained on the three screens and pan and this has been used to evaluate chewing activity and mean rumen pH . A consistent method to estimate peNDF in dairy rations on dairy farms is still to be determined.
It is generally recognized that rumen particles larger than 1.18 mm are large particles and are retained in the rumen. However, Kononoff and Heinrichs (2003a,b) and Maulfair and Heinrichs (2010) found particles larger than those retained on a 1.18 mm screen consistently in cows consuming various forage NDF sources. It may be that cows consuming higher dry matter amounts from wet forages will pass larger particles than observed by Poppi (1980) who investigated fecal particles in cattle consuming dry hay diets at lower dry matter intakes. In point of fact, fecal particles correspond to the size of particles leaving the rumen as little reduction in size occurs in the distal gastro-intestinal tract. Possibly fecal particle size should be included in an assessment of dietary particle size to more accurately characterize peNDF for dairy cattle.
Most studies investigating particle size have used silages with chopped hay as forage components. In Sicily forages on farms typically are a mixture of long hay and silage, either triticale or corn silage. The hay is usually mature, greater than $60 \%$ NDF and $10 \%$ or less in CP, and is harvested once in May. In herds that feed a TMR, hay is added into the mixer wagon as long material, resulting in longer particle size than typical for chopped or ground hays. Concentrate mixes are usually a composite of ground corn and at times some barley grain, soybean meal (44\%) or sunflower meal, beet pulp, a rumen protected fat, and minerals and vitamins. Some farms will supplement with fresh citrus pulp, but this is only available January
through April. Flaked soy beans and wheat bran may also be included in concentrate mixes but these are not used routinely. Particle size of TMR tends to be coarse and total NDF tends to be higher than observed on US dairy farms.
The objective of this study was to assess the association of particle size in TMR estimated using the PSPS with production of milk, fat and protein on a convenience sample of Ragusa dairy farms, while controlling for nutrient content.

### 5.2 MATERIALS AND METHODS

## Data

Production data for milk (kg), fat (g), and protein (g) and TMR information were collected from 25 herds located in Ragusa province (Italy) from 2006 through 2008 and formed a dataset including 46,531 test-day (TD) records from 3,554 cows. This dataset was used to estimate association of random individual curves for milk yield with particle size distribution of the diets. To estimate variance components for the genetic effects more precisely, a larger dataset (full dataset) with more animals than the ones with known TMR was necessary. A full dataset including 241,153 TD records from 9,809 animals in 42 herds recorded from 1995 through 2008 was supplied by the local milk recording agency (APA Ragusa, Italy) and used to estimate variance components for milk ( kg ), fat ( g ), and protein ( g ) yield using a random regression TD model.
For the 25 herds included in the reduced dataset, TMR samples were collected every 3 months from March 2006 through December 2008, sieved through the PSPS according to the procedure described by Heinrichs and Kononoff (2002), and analyzed for ash (AOAC, 1994), crude protein (CP, AOAC, 1994), soluble nitrogen (SN, Licitra et al., 1996), acid detergent lignin (ADL, Goering and Van Soest, 1970), neutral detergent fiber (NDF, Van Soest et al., 1991), acid detergent fiber (ADF, Goering and Van Soest, 1970), and starch (AOAC 1998, method 996.11). All chemical analyses were expressed on a DM basis. Diets were also evaluated using CPM Dairy (version 3.0.8, University of Pennsylvania, Kennett Square, PA, Cornell University, Ithaca, NY and Miner Agricultural Research Institute, Chazy, NY). Residues on the 3 sieves ( 19 mm , upper; 8 mm , middle; 1.18 mm , lower) and the bottom content were weighed and proportions on total weight were calculated on as fed basis. Afterwards, peNDF was calculated as the proportion of TMR retained on the top ( 19 mm ) and middle ( 8 mm ) screen times the ration NDF content (Yang et al., 2001). The mean geometric particle length was calculated based on the proportion of particles greater than 8 mm in size (Armentano and Tayson, 2005).

## Particle Size Classes Identification

Overall means and standard deviations for particle distribution on the four PSPS sieves and nutrient content were calculated (Table 5.1). In addition, ranges for nutrient content and particles on the upper and middle PSPS sieves were plotted and examined for uniformity of distribution. Penn State recommends <= $8 \%$ of TMR particles be collected on the upper screen of the particle separator. Due to the distribution of upper sieve particles in this data set, categories of <10.4\%, 10.4 to $17.4 \%$, and $>17.4 \%$ were made. Secondly, peNDF recommendations are based on the sum of the top two screens and PSU recommends that 30 to $50 \%$ of particles be on the middle and lower sieves and up to $20 \%$ of particles be on the bottom sieve. Given an upper maximum of $10.4 \%$ and a bottom maximum of $20 \%$, then $34.9 \%$ for the middle and lower screen are allowable solutions. Therefore the middle screen was classified based on <30.1, 30.1 to 35.6 , and $>35.7$. This resulted in nine classes of sieve categories based on top screen and middle screen classifications.

Table 5.1 Mean dietary composition and Penn State Particle distribution for 25 farms in the Ragusa region of Sicily.

| Item, | \% DM | SD | Min | Max |
| :--- | :---: | :--- | :---: | ---: |
| Crude protein | 15.16 | 1.53 | 11.05 | 18.57 |
| Soluble protein, \% CP | 32.75 | 6.84 | 11.17 | 60.55 |
| Neutral Detergent Fiber | 40.69 | 4.26 | 25.01 | 50.07 |
| Acid Detergent Fiber | 23.78 | 3.16 | 13.71 | 32.07 |
| Starch $_{\text {Nonfiber carbohydrate }}{ }^{1}$ | 19.69 | 3.01 | 4.02 | 7.58 |
| Acid Detergent Lignin | 4.21 | 1.05 | 22.07 | 32.26 |
| Ether Extract | 4.28 | 1.21 | 1.04 | 49.89 |
| Ash | 7.99 | 0.85 | 2.70 | 8.43 |
|  |  |  | 6.08 | 7.27 |

Proportion on Penn State Particle Separator, \%

| Top Screen 19.0 mm | 14.70 | 8.05 | 1.36 | 38.77 |
| :--- | ---: | ---: | ---: | ---: |
| Middle Screen 9.0 mm | 33.66 | 7.78 | 13.07 | 58.50 |
| Lower Screen 1.6 mm | 35.45 | 6.00 | 20.42 | 49.07 |
| Bottom pan $_{\text {peNDF }^{2}}$ | 16.19 | 5.02 | 1.91 | 28.08 |
| GMPL $^{3}$ | 19.79 | 4.88 | 10.81 | 33.50 |

${ }^{1}$ Calculated as 100 - CP - NDF - EE - Ash
${ }^{2}$ peNDF calculated as material retained on top two screens times NDF in ration (Yang et al., 2001)
${ }^{3}$ GMPL $=$ geometric mean particle length (Armentano and Tayson, 2005)
GMPL $=0.54+11.84$ * (proportion $>9 \mathrm{~mm}$ in TMR)

## Association of Particle Size Classes with Feed Compositions and Production

To examine differences in CP, NDF, starch, ash, acid detergent lignin (ADL), ADF, and ether extract (EE) across particle size classes, the following mixed model, with herd nested within class as the repeated term with covariance matrix set to autoregressive(1), was applied using SAS statistical software (version 9.1.3, SAS Institute, Inc., Cary, NC):

$$
Y_{j}=u_{i}+\text { class }_{j}+\text { herd }_{k}\left(\text { class }_{j}\right)+e_{j k l}
$$

where:
$Y_{j}=$ mean of interest for PSPS, CP, NDF, starch, ash, ADL, ADF and EE;
$u_{i}=$ overall mean for the ith item;
class $_{j}=$ jth class based on upper and middle sieve categories (1 to 9);
herd $_{k}\left(\right.$ class $\left._{\mathrm{j}}\right)=$ kth herd nested in the jth particle size class ( 25 herds);
$\mathrm{e}_{\mathrm{jkl}}=$ residual, random error.

Actual test day records for milk and milk component yields across particle size classes were examined using the above model with cow nested within herd as a repeated effect.

## Association of Particle Size Classes with Individual Lactation Curves

In order to estimate particle size class effect on individual lactation curves, a multiplelactation, single-trait random regression TD model was fitted to the production data combined with the TMR particle size information coming from the 25 farms involved in the study. Since TD records were collected monthly whereas TMR were sampled every 3 months, each TD record was associated to the closest TMR fed to animals immediately before or after the test day. Prior to this analysis, variance components for the genetic effects were estimated using a larger full dataset of production data from 9,809 animals in 42 herds. Production TD records for the full dataset were processed using a multiplelactation, single-trait random regression TD model, with age at calving $\times$ year of calving, parity $\times$ stage of pregnancy, year of test $\times$ month of test set as fixed effects, and herd $\times$ test date, sire and maternal grandsire additive genetic effect, and permanent environmental effect set as random effects modeled using third-order Legendre polynomials (Caccamo et al. 2012). Model fitting was carried out using ASREML (Gilmour et al. 2009). Estimated variance components were then used to investigate the effect of TMR particle size classes on milk, fat, and protein yield lactation curves. An extension of the model used for variance
components estimation was fitted, including TMR particle size class effect modeled as an interaction with DIM using a ninth-order Legendre polynomial to increase the sensitivity to dietary effects across DIM. To account for TMR chemical composition, CP, NDF, and starch and their interaction with DIM (fitted as a ninth-order Legendre polynomial) were also included in the model. Significance of effects was tested using the conditional Wald Fstatistic (Gilmour et al. 2009).
Parameters estimated in the model were used to generate lactation curves for milk, fat, and protein yield for each class of TMR particle size (Gilmour et al. 2004). In order to have purely estimates of particle distribution not confounded by nutrient content of TMR, predictions of lactation curves per particle size class were controlled for TMR chemical composition. As the range in nutrient content of the TMR within class overlapped sufficiently, it was determined that production data could be controlled for CP content of $15.5 \%$, NDF of $40.7 \%$ and starch of $19.7 \%$ for all classes. Mean production values for predicted lactation curves were tested for differences across particle size classes.

### 5.3 RESULTS AND DISCUSSION

## Chemical Characteristics of Diets

A total of 148 TMR samples with sieve and composition analysis were available from 25 farms. Table 5.1 presents the mean ration composition and PSPS distributions for the 25 farms. Across all TMR samples, mean CP, NDF, and starch content were $15.16 \%, 40.69 \%$, and $19.69 \%$, respectively (Table 5.1). Although there were differences in the mean CP, starch, and NDF content between the classes, the range in nutrient content of the TMR within class overlapped sufficiently that it was determined that prediction of individual production curves could be controlled for CP content of $15.5 \%$, NDF of $40.7 \%$ and starch of $19.7 \%$ for all classes to assess the influence of particle distribution on milk production. Therefore the production effects in the paper are purely estimates of particle distribution not confounded by nutrient content of TMR.

As seen in Table 5.2, there were differences in the least square mean nutrient content and particle distributions for the classes. Of all major nutrients, CP varied the most across the particle size classes and there were only trends for differences in NDF and starch content ( $\mathrm{P}<0.09$ ). In general, the diets which had the greatest proportion of TMR on the upper sieve had the greatest mean values for NDF, the lowest CP and starch content of the TMR samples. The content of NDF and starch would be expected to only support a moderate level of milk production, 25 to 35 kg
of milk, depending on dry matter intake. With NDF content of $40.69 \%$ and given estimates of maximal NDF intake of $1.3 \%$ of body weight, maximal dry matter intake would be 19.2 to $20.8 \mathrm{~kg} / \mathrm{d}$ for body sizes of 600 to 650 kg . Approximate intake on Ragusa dairy farms is approximately 20 to $21 \mathrm{~kg} / \mathrm{d}$ (G. Azzaro, personal observation), so this seems to be reasonable given the NDF content of the diets. Mertens (1997) suggests a maximal intake of $1.05 \%$ of peNDF, but given the peNDF estimate of 19.79 based on the PSPS particle distribution, this would correspond to maximal intakes of 31 to $35 \mathrm{~kg} / \mathrm{d}$, an amount unlikely in Ragusa. Of course milk production also influences dry matter intake, as does body size and days in milk (NRC, 2001), therefore milk production may also be a factor limiting intakes in Ragusa.

## Physical Characteristics of Diets

The partitioning of diets into classes based on the top two PSPS screens in presented in Table 5.2. Yang and Beauchemin (2007) observed that chewing activity most correlated with the proportion of material retained on the top two sieves of the PSPS. The proportion of the TMR on the upper, middle, lower and bottom differed across the classes, as expected by the classification scheme. Penn State extension recommends that the upper screen contain $2-8 \%$ of TMR, which was the basis of choosing <10.4 as the first cut-off for the TMR classes. Secondly it is recommended that the middle sieve contain 30 to $50 \%$ of the TMR. We chose to categorize the middle screen based on less than $30.1 \%$, the too short recommendation in the PSU classification, and by 30 to $35.3 \%$, an intermediate range, and $>35.6 \%$. Since it recommended that the bottom pan contain up to $20 \%$ feed material and the middle and lower screens contain $30 \%$ to $50 \%$ of feed material, when the top screen cut point is set to $10 \%$, to meet these guidelines, the middle and lower sieves must each retain $35 \%$ of the feed material. Thus $35.6 \%$ was chosen as the boundary for defining the middle screen categories.
For classes 1 to 3 the lower and bottom sieve proportions were fixed at $39.0 \%$ and $18.1 \%$, respectively. For classes 4 to 6 , the lower and bottom sieve values were set to $35.0 \%$ and $16.2 \%$ and for classes 7 to 8 the lower and bottom sieve proportions were set to $32.2 \%$ and $14.3 \%$. These were mean values for the sieve proportions within these classes.
5 - Diet particle size effect on individual lactation curves

| Screen mm, \% |  |  |  |  |  |  |  |  |  | PSPS ${ }^{2}$ Sieve \% Retained- |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\mathrm{Cl}^{1}$ | Top | Middle | N | CP | sem | NDF | sem | Starch | sem | Upper sem | Middle | sem | Lower | sem | Bottom | sem |
| 1 | <10.4 | < 30.1 | 10 | $16.20^{\text {a }}$ | 0.52 | $39.76^{\text {ac }}$ | 1.29 | $22.11^{\text {a }}$ | 1.06 | $7.2^{\text {a }} 1.3$ | $27.2^{\text {a }}$ | 1.4 | $44.0^{\text {a }}$ | 1.5 | $21.6{ }^{\text {a }}$ | 1.3 |
| 2 | <10.4 | 30.1-35.6 | 15 | $15.95{ }^{\text {a }}$ | 0.45 | $38.05{ }^{\text {c }}$ | 1.06 | $19.64{ }^{\text {ab }}$ | 0.88 | $6.4{ }^{\text {a }} 1.2$ | $33.6{ }^{\text {b }}$ | 1.2 | $40.7{ }^{\text {ab }}$ | 1.3 | $19.3{ }^{\text {a }}$ | 1.1 |
| 3 | <10.4 | >35.6 | 23 | $15.05^{\text {ab }}$ | 0.48 | $39.83^{\text {ac }}$ | 0.85 | $20.26^{\text {ab }}$ | 0.70 | $6.5^{\text {a }} 0.9$ | $41.1^{\text {c }}$ | 0.9 | $36.2{ }^{\text {bc }}$ | 1.0 | $16.2^{\text {b }}$ | 0.9 |
| 4 | 10.4-1 | 17.4 <30.1 | 15 | $14.46^{\text {b }}$ | 0.48 | $40.57^{\text {acb }}$ | 1.06 | $20.92{ }^{\text {ab }}$ | 0.89 | $14.4{ }^{\text {b }} 1.3$ | $25.8{ }^{\text {a }}$ | 1.2 | $39.0{ }^{\text {b }}$ | 1.4 | $20.8{ }^{\text {a }}$ | 1.2 |
| 5 | 10.4-1 | 17.4 30.1-35.6 | 12 | $16.10^{\text {a }}$ | 0.48 | $39.12^{\text {ac }}$ | 1.18 | $18.82{ }^{\text {bc }}$ | 0.97 | $13.7{ }^{\text {b }} 1.2$ | $32.4{ }^{\text {b }}$ | 1.3 | $35.1{ }^{\text {cd }}$ | 1.4 | $18.7{ }^{\text {ab }}$ | 1.2 |
| 6 | 10.4-1 | $17.4>35.6$ | 23 | $15.26^{\text {ab }}$ | 0.36 | $40.05^{\text {ac }}$ | 0.85 | $20.51{ }^{\text {ab }}$ | 0.71 | $12.7{ }^{\text {b }} 0.9$ | $40.4{ }^{\text {c }}$ | 1.0 | $33.9{ }^{\text {cd }}$ | 1.1 | $12.9{ }^{\text {c }}$ | 0.9 |
| 7 | >17.4 | <30.1 | 22 | $14.31{ }^{\text {b }}$ | 0.37 | $42.86{ }^{\text {b }}$ | 0.87 | $18.53^{\text {c }}$ | 0.72 | $25.9{ }^{\text {c }} 1.0$ | $24.1{ }^{\text {a }}$ | 1.0 | $33.8{ }^{\text {cd }}$ | 1.1 | $16.1{ }^{\text {b }}$ | 0.9 |
| 8 | >17.4 | 30.1-35.6 | 15 | $15.08{ }^{\text {ab }}$ | 0.40 | $42.12^{\text {ab }}$ | 1.05 | $19.55^{\text {ab }}$ | 0.84 | $218^{\text {c }} 1.0$ | $32.6{ }^{\text {b }}$ | 1.1 | $32.0{ }^{\text {d }}$ | 1.2 | $13.7{ }^{\text {bc }}$ | 1.0 |
|  | >17.4 | >35.6 | 13 | $14.30^{\text {b }}$ | 0.47 | $43.45{ }^{\text {b }}$ | 1.13 | $17.04{ }^{\text {c }}$ | 0.93 | $23.2{ }^{\text {c }} 1.2$ | $41.7{ }^{\text {c }}$ | 1.2 | $25.6{ }^{\text {e }}$ | 1.4 | $9.5{ }^{\text {d }}$ | 1.2 |

${ }^{1} \mathrm{Cl}=$ class
${ }^{2}$ PSPS $=$ Penn State Particle Separator, Kononoff et al. 2003
Means with different superscript within column differ by $P<0.05$
Means with different superscript within column differ by $P<0.05$
Main effects of starch and NDF had trends at $P<0$

In general nutritionists are concerned when the upper sieve contains more than $10 \%$ material due to sorting that may occur when this is excessive. Maulfair et al. (2010) observed that cows ate less of chopped grass hay when $11.7 \%$ of the particles were retained on 26.9 mm sieve compared with $8.61 \%$ or lower. Thus cows were very selective with just a small increment in particle size. If intake of longer particles decreased then intake of shorter particles increased, particularly of particles in the bottom pan (Maulfair et al., 2010). The effect due to sorting was that cows ate less NDF and more starch than offered in the TMR when more long particles were present in the diet.
PSU recommends that less than 20\% of the TMR be in the bottom pan. Classes 1 and 4 (Table 5.2) had least square mean values of more than $20 \%$ in the bottom pan. Class 1 diets had the lowest least square mean GMPL, 4.64 mm (sem 0.19) whereas class 2 ( 5.28 mm , sem 0.17 ) and class $4(5.32 \mathrm{~mm}$, sem 0.18 ) had the next lowest mean GMPL estimates. All other class had least square mean GMPL from 6.00 to 8.22 mm (data not shown). The authors are not aware of a recommendation for a mean GMPL score for a TMR. General recommendations are that the diet contains $19 \%$ to $21 \%$ peNDF on a DM basis or that forage NDF comprises $75 \%$ or more of total NDF (NRC, 2001). The overall mean value for peNDF was 19.78 within NRC (2001) ranges. However, peNDF for class 1 TMR was 13.67 (sem 0.93) and only over $21 \%$ for classes 6 through 9 (Table 5.3). Least square means for GMPL are presented in table 5.3. As for peNDF the lowest mean GMPL was for diets of class 1 and the longest mean GMPL was for diets from class 6 and greater. Class 3 and class 5 diets were intermediate in length between the shortest three classes, class 1, 2 and 4, and the longest classes, class 6 and higher (Table 5.3).

Table 5.3 Least square mean values for geometric mean particle length and peNDF based on particle size classes for the top two PSPS sieves.

| Screen mm, PSPS ${ }^{1} \%$ |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Class | Top | Middle | peNDF ${ }^{2}$ | sem | GMPL ${ }^{3}$ | sem |
| 1 | <10.4 | < 30.1 | $13.66{ }^{\text {a }}$ | 1.08 | $4.61{ }^{\text {a }}$ | 0.23 |
| 2 | <10.4 | 30.1-35.6 | $15.41{ }^{\text {a }}$ | 0.94 | $5.27{ }^{\text {b }}$ | 0.19 |
| 3 | <10.4 | >35.6 | $18.88{ }^{\text {c }}$ | 0.75 | $6.17{ }^{\text {c }}$ | 0.15 |
| 4 | 10.4-17.4 | <30.1 | $16.23{ }^{\text {ab }}$ | 0.98 | $5.29{ }^{\text {b }}$ | 0.20 |
| 5 | 10.4-17.4 | 30.1-35.6 | $18.04{ }^{\text {bc }}$ | 1.02 | $6.00{ }^{\text {c }}$ | 0.21 |
| 6 | 10.4-17.4 | >35.6 | $21.46{ }^{\text {d }}$ | 0.76 | $6.84{ }^{\text {de }}$ | 0.16 |
| 7 | >17.4 | <30.1 | $21.55{ }^{\text {d }}$ | 0.78 | $6.47{ }^{\text {cd }}$ | 0.16 |
| 8 | >17.4 | 30.1-35.6 | $22.99^{\text {d }}$ | 0.86 | $6.97{ }^{\text {e }}$ | 0.17 |
| 9 | >17.4 | >35.6 | $28.18{ }^{\text {e }}$ | 0.99 | $8.20{ }^{\text {f }}$ | 0.20 |

${ }^{1}$ PSPS $=$ Penn State Particle Separator, Kononoff et al. 2003
${ }^{2}$ peNDF = sum of TMR particles collected on the upper ( 19 mm ) and middle ( 9 mm ) sieves times the NDF content of the TMR (Yang et al, 2001)
${ }^{3}$ GMPL $=$ geometric mean particle length calculated based on Armentano and Tayson (2005) as GMPL, $\mathrm{mm}=0.54+11.84 \times$ (proportion particles of TMR retained $>9 \mathrm{~mm}$ ) Means with different superscript within column differ by $P<0.05$

## Particle size class effect on herd level milk and milk components yield

Mean herd level production for milk, fat, and protein of the 25 herds included in this study are presented in Table 5.4. In general, for all traits, production was lowest for middle screen proportion $>35.6$ within each top screen proportion class (Classes 3, 6, 9 in Table 5.4). Differences were not significantly different, but within the three groupings based on the retention of material on the top screen, peNDF was greatest when material retained on the middle screen was $>35.6 \%$ of the TMR. Within each class more material retained on the middle screen resulted in less fine material retained on the lower screen and passing to the pan. These rations had longer mean GMPL and were coarser than the other diets within each class.

Table 5.4 Least square means for milk, fat and protein yield for the nine particle size classes used in this study.

| Class | Screen mm, \% |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | Top | Middle | Milk | sem | Fat sem | Protein sem |
| 1 | <10.4 | < 30.1 | $29.30^{\text {a }}$ | 0.10 | $1102.47{ }^{\text {d }} 3.98$ | $1032.74{ }^{\text {bc }} 4.45$ |
| 2 | <10.4 | 30.1-35.6 | $34.59{ }^{\text {b }}$ | 0.14 | $1179.82^{\text {b }} 5.57$ | $1056.95^{\text {a }} 4.71$ |
| 3 | <10.4 | >35.6 | $27.58{ }^{\text {e }}$ | 0.21 | $1008.00{ }^{\text {f }} 8.13$ | $990.90^{\text {e }} 6.5$ |
| 4 | 10.4-17.4 | <30.1 | $35.69{ }^{\text {a }}$ | 0.10 | $1241.33^{\text {a }} 4.00$ | $1017.84{ }^{\text {d }} 4.46$ |
| 5 | 10.4-17.4 | 30.1-35.6 | $32.65{ }^{\text {c }}$ | 0.14 | $1109.11^{\text {d }} 5.43$ | $1009.74{ }^{\text {d }} 4.85$ |
| 6 | 10.4-17.4 | >35.6 | $32.93{ }^{\text {c }}$ | 0.15 | $1139.50{ }^{\text {c }} 6.06$ | $1013.55{ }^{\text {d }} 6.82$ |
| 7 | $>17.4$ | <30.1 | $30.45{ }^{\text {d }}$ | 0.15 | $1065.56{ }^{\text {e }} 5.73$ | $1054.46{ }^{\text {a }} 5.39$ |
| 8 | >17.4 | 30.1-35.6 | $30.18{ }^{\text {d }}$ | 0.14 | $1074.07^{\text {e }} 5.58$ | $1034.08^{\text {b }} 4.46$ |
| 9 | >17.4 | >35.6 | $27.35{ }^{\text {e }}$ | 0.12 | $964.00^{\text {g }} 4.86$ | $1020.39{ }^{\text {cd }} 5.08$ |

Means with different superscript within column differ by $P<0.05$

## Particle size class effect on individual lactation curves

Figures 5.1, 5.2 and 5.3 present the influence of particle proportion classes on milk yield (kg/d, Figure 5.1), fat yield (g/d, Figure 5.2), and protein yield (g/d, Figure 5.3). There was little effect of class of particle proportions on the top two sieves and peNDF on milk yield and fat yield. In general, as seen in table 5.5 , milk yield and fat yield tended to be slightly greater as class increased from class 1 to class 9. Proportion of particles retained on the top two PSPS sieves that were associated with peNDF greater than $21.0 \%$ of dry matter had the highest milk and fat yields. However, class 4, with particle retention on the upper sieve of $10.4 \%$ to $17.4 \%$ and middle screen of $<30.1 \%$, and a peNDF of 15.95 had high milk yield and fat yield compared to other yields in classes 1 to 3 and classes 4 and 5 (Table 5.5). Overall effects were minor on milk yield and fat yield so trends should be viewed cautiously.
Of interest is the effect of sieve classes on protein yield. Protein yield was significantly greater for sieve classes 4,5 , and 6, all sieve classes with 10.4 to $17.4 \%$ of TMR particles retained on the upper PSPS 19 mm sieve (Table 5.5, and Figure 5.3). Sieve classes which retained more than $17.4 \%$ of particles on the upper sieve had the lowest protein yields. In this data, protein yields were enhanced when the upper sieve contained 10.4 to $17.4 \%$ particles irrespective of the middle sieve proportions. Dietary CP was $15.5 \%$, starch was $19.7 \%$ and NDF was $40.7 \%$ for the analysis. This result was surprising. Only Kononoff and Heinrichs (2003a, 3003b) have observed an influence of particle size on protein content in milk. Most studies examining particle size have not found an effect on milk protein yield or content
(Krause, 2002; Beauchemin et al., 2003; Krause and Combs, 2003; Bhandari et al., 2007; Bhandari et al., 2008). Since nutrient content was controlled in the analysis, we may speculate that sieve classes with $10.4 \%$ to $17.4 \%$ particles on the top screen, were associated with the most uniform intake of nutrients resulting in an influence on protein yield in milk. Within this class of particle sizes (Class 4, 5, and 6; Table 5.2), the lower screen and pan had approximately $59.8 \%, 53.8 \%$ and $46.8 \%$ of TMR material. TMR in classes 1,2 , and 3 had $65.6 \%, 60.0 \%$ and $52.0 \%$ of material on the lower screen and pan, whereas classes 7, 8 and 9 had 49.9\%, 45.7\% and $35.1 \%$ of material on the lower screen and pan. Therefore, the middle classes, 4, 5 and 6, had the most consistent distribution of fine particles relative to the PSU recommendations of 30 to $50 \%$ of material on the lower screen and $20 \%$ of material in the pan. This may have resulted in the most uniform intake of nutrients of the three classes.


Figure 5.1 Milk production curves for classes of TMR categorized by particle retention on the Penn State Particle Separator. Diets are categorized into nine classes based on proportion of particles retained on the upper sieve, 19 mm , and the middle sieve, 9 mm . Dashed curves are for one-component model for classes of particle separation, solid curves multicomponent models including covariates for mean CP concentration of TMR, $15.5 \%$, starch, $19.7 \%$ and NDF, $40.7 \%$ and their interaction with days in milk.


Figure 5.2 Fat production curves for classes of TMR categorized by particle retention on the Penn State Particle Separator. Diets are categorized into nine classes based on proportion of particles retained on the upper sieve, 19 mm , and the middle sieve, 9 mm . Dashed curves are for one-component model for classes of particle separation, solid curves multicomponent models including covariates for mean CP concentration of TMR, 15.5\%, starch, $19.7 \%$ and NDF, $40.7 \%$ and their interaction with days in milk.


Figure 5.3 Protein production curves for classes of TMR categorized by particle retention on the Penn State Particle Separator. Diets are categorized into nine classes based on proportion of particles retained on the upper sieve, 19 mm , and the middle sieve, 9 mm . Dashed curves are for one-component model for classes of particle separation, solid curves multi-component models including covariates for mean CP concentration of TMR, 15.5\%, starch, $19.7 \%$ and NDF, $40.7 \%$ and their interaction with days in milk.

Table 5.5 Mean production values based on curves in figures 5.1, 5.2, and 5.3 for outputs from the test day model controlling for 9 classes of particle size based on particles in TMRs retained on the top two Penn State Particle Separator sieves ( 19 mm and 8 mm ).

| Class | peNDF | Milk, kg/d | sem | Fat, g/d | sem | Protein, g/d | sem |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 1 | 13.67 | 38.04 | 0.02 | 1244.5 | 1.3 | 1165.2 | 1.8 |
| 2 | 15.91 | 38.00 | 0.02 | 1241.4 | 1.3 | 1162.1 | 1.8 |
| 3 | 18.73 | 37.96 | 0.02 | 1238.2 | 1.3 | 1159.0 | 1.8 |
| 4 | 15.95 | 38.22 | 0.02 | 1248.1 | 1.3 | 1248.1* | 1.8 |
| 5 | 18.00 | 38.18 | 0.02 | 1245.0 | 1.3 | 1245.0* | 1.8 |
| 6 | 21.88 | 38.15 | 0.02 | 1241.9 | 1.3 | 1241.9* | 1.8 |
| 7 | 21.20 | 38.35 | 0.02 | 1249.6 | 1.3 | 1158.2 | 1.8 |
| 8 | 23.35 | 38.31 | 0.02 | 1246.5 | 1.3 | 1155.2 | 1.8 |
| 9 | 28.15 | 38.27 | 0.02 | 1243.4 | 1.3 | 1152.1 | 1.8 |

Class 1 Upper Sieve Proportion Particles: <10.4, Middle Sieve <30.1
Class 2 Upper Sieve Proportion Particles: <10.4, Middle Sieve 30.1-35.6
Class 3 Upper Sieve Proportion Particles: <10.4, Middle Sieve > 35.6
Class 4 Upper Sieve Proportion Particles: 10.4-17.4, Middle Sieve <30.1
Class 5 Upper Sieve Proportion Particles: 10.4-17.4, Middle Sieve 30.1-35.6
Class 6 Upper Sieve Proportion Particles: 10.4-17.4, Middle Sieve > 35.6
Class 7 Upper Sieve Proportion Particles: >17.4, Middle Sieve <30.1
Class 8 Upper Sieve Proportion Particles: >17.4, Middle Sieve 30.1-35.6
Class 9 Upper Sieve Proportion Particles: >17.4, Middle Sieve > 35.6

### 5.4 CONCLUSION

In conclusion, based on this study, particle size distribution in Ragusa dairy farms TMR were associated with small but significant effects on milk protein yield. The distribution most associated with increased protein yield was when the top PSPS screen contained $10.4 \%$ to $17.4 \%$ of TMR particles and the lower screen and pan contained $45 \%$ to $59 \%$ of TMR particles. Optimal distributions were different than those recommended by PSU extension and may reflect regional differences based on types of forages fed.

## REFERENCES

AOAC, 1994. Official methods of analysis (15th edition), Association of Official Analytical Chemists, Arlington, VA, USA.
AOAC, 1998. Official methods of analysis of AOAC International: 4th revision. 16th ed. Arlington, Va.: Assoc of Official Analytical Chemists Intl.

Armentano, L.E. and D. Taysom 2005. Short Communication: Prediction of Mean Particle Size and Proportion of Very Long Fiber Particles from Simplified Sieving Results. J. Dairy Sci. 88:3982-3985.
Bhandari, S.K., K. H. Ominski, K. M. Wittenberg, and J. C. Plaizier. 2007. Effects of Chop Length of Alfalfa and Corn Silage on Milk Production and Rumen Fermentation of Dairy Cows. J. Dairy Sci. 90:2355-2366.
Bhandari, S.K., S. Li, K. H. Ominski, K. M. Wittenberg, and J. C. Plaizier. 2008. Effects of the Chop Lengths of Alfalfa Silage and Oat Silage on Feed Intake, Milk Production, Feeding Behavior, and Rumen Fermentation of Dairy Cows. J. Dairy Sci. 91:1942-1958.
Beauchemin, K. A., W. Z. Yang, and L. M. Rode. 2003. Effects of particle size of alfalfa-based dairy cow diets on chewing activity, ruminal fermentation, and milk production. J. Dairy Sci. 86:630-643.
Caccamo, M., R. F. Veerkamp, G. Licitra, R. Petriglieri, F. La Terra, A. Pozzebon, and J. D. Ferguson. 2012. Association of total-mixed-ration chemical composition with milk, fat, and protein yield lactation curves at individual level. J. Dairy Sci. TBC:113 http://dx.doi.org/10.3168/jds.2011-4148.
Gilmour, A., B. Cullis, S. Welham, B. Gogel, and R. Thompson. 2004. An efficient computing strategy for prediction in mixed linear models. Computational Statistics \& Data Analysis 44:571-586.
Gilmour, A. R., B. J. Gogel, B. R. Cullis, and R. Thompson. 2009. "ASReml User Guide". NSW Department of Primary Industries, Orange, Australia.
Goering, H. K., and P. J. Van Soest. 1970. Forage fiber analysis (apparatus, reagent, procedures and some applications). US Department of Agriculture, Agriculture Handbook No. 379.
Grant, R. J., and V. F. Colenbrander. 1990a. Milk fat depression in dairy cows: Role of particle size of alfalfa hay. J. Dairy Sci. 73:1823-1833.
Grant, R. J., and V. F. Colenbrander. 1990b. Milk fat depression in dairy cows: Role of silage particle size. J. Dairy Sci. 73:1834-1842.
Heinrichs, A. J., and P. J. Kononoff. 2002. Evaluating particle size of forages and TMRs using the New Penn State Forage Particle Separator. Cooperative Extension, College Agric. Sci. 42:1-15.
Kononoff, P. J., and A. J. Heinrichs. 2003a. The effect of corn silage particle size and cottonseed hulls on cows in early lactation. J. Dairy Sci. 86:2438-2451.
Kononoff, P. J., and A. J. Heinrichs. 2003b. The effect of reducing alfalfa haylage particle size on cows in early lactation. J. Dairy Sci. 86:1445-1457.

Kononoff, P. J., A. J. Heinrichs, and D. R. Buckmaster. 2003. Modification of the Penn State forage and total mixed ration particle separator and the effects of moisture content on its measurements.J. Dairy Sci. 86:1858-1863.
Krause, K. M., and D. K. Combs. 2003. Effects of forage particle size, forage source, and grain fermentability on performance and ruminal pH in midlactation cows. J. Dairy Sci. 86:1382-1397.
Krause, K. M., D. K. Combs, and K. A. Beauchemin. 2002a. Effects of forage particle size and grain fermentability in midlactation cows. I. Milk production and diet digestibility. J. Dairy Sci. 85:1936-1946.
Lammers, B. P., D. R. Buckmaster, and A. J. Heinrichs. 1996. A simple method for the analysis of particle sizes of forage and total mixed rations. J. Dairy Sci 79:922928.

Licitra, G., T. M. Hernandez, and P. J. Van Soest. 1996. Standardization of procedures for nitrogen fractionation of ruminant feeds. Anim. Feed Sci. Technol. 57:347-358.
Maulfair, D.D., A.J. Heinrichs. 2010. Technical note: Evaluation of procedures for analyzing ration sorting and rumen digesta particle size in dairy cows. J. Dairy Sci. 93:3784-3788.
Maulfair, D.D., G.I. Zanton, M. Fustini, and A.J. Heinrichs. 2010. Effect of feed sorting on chewing behavior, production, and rumen fermentation in lactating dairy cows. J. Dairy Sci. 93 :4791-4803.

Mertens, D.R. 1997. Creating a system for meeting the fibre requirements of dairy cows. J. Dairy Sci. 80:1463-1481.

NRC. 2001. Nutrient Requirements of Dairy Cattle. 7th Rev. Ed. National Research Council. Nat. Acad. Sci., Washington, DC.

Schadt, I., J.D. Ferguson, G. Azzaro, R. Petriglieri, M. Caccamo, P. Van Soest , and G. Licitra. 2012. How do dairy cows chew?—Particle size analysis of selected feeds with different particle length distributions and of respective ingested bolus particles. J. Dairy Sci. 95:4707-4720.
Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583-3597.
Woodford. S. T., and M. R. Murphy. 1988. Dietary alteration of particle breakdown and passage from the rumen in lactating dairy cattle. J. Dairy Sci. 71:687.
Yang, W.Z. and K. A. Beauchemin. 2007. Altering physically effective fiber intake through forage proportion and particle length: chewing and ruminal pH. J. Dairy Sci. 90:2826-2838

Yang, W. Z., K. A. Beauchemin, and L. M. Rode. 2001. Effects of grain processing, forage to concentrate ratio, and forage particle size on rumen pH and digestion by dairy cows. J. Dairy Sci. 84:2203-2216.

## 6

General discussion

### 6.1 INTRODUCTION

Milk recording is recognized as a valuable tool for herd management worldwide. However, proper analyses and data handling are necessary to derive accurate and unbiased information to analyze herd/cow performance. An accurate unbiased analysis of milk production data can provide farmers with a tool that support them in managing their herds to improve milk production and quality.
To evaluate management practices and to develop management tools, a field study was conducted in Southern Italy (Ragusa province) to collect information at herd level (every 3 months) and test records at individual cow level (every month). Data collection was performed from March 2006 through December 2008 on 40 cooperating farms.
In this thesis, first, benefits of using a random regression test-day (TD) model for management improvement (Chapter 2) are clearly shown. Higher variance of the lactation curves at herd level (between herds) compared to the phenotypic variance (between animals) around lactation peak, suggest that development of management parameters for milk, fat, and protein yield around the peak should focus on between herd parameters rather than management parameters that compare individual cows, arguing the possibility to use herd curves as a tool to evaluate and improve management between herds. In Chapter 3 sources of variation that explain herd curve were explored, and breed, feeding systems and total-mixed-ration (TMR) chemical composition were shown to influence herd curve peak, mean and persistency. Herd curves therefore are useful to inform farmers about inappropriate feeding, after correcting properly for breed and feeding system. The response in milk and milk components production to varying diet composition was then investigated at individual cow level. The analyses were performed by combining TD information with chemical composition (Chapter 4) and particle size (Chapter 5) of TMR fed to animals. A different approach compared to most experiments investigating production responses to varying nutritional quality was developed: the field study approach allowed a retrospective explanation of variation in lactation curves due to diet chemical and physical composition on a very large dataset including a large number of herds and animals. In this chapter, the contribution of the analyses performed in this thesis to development of management parameters derived from milk recording system information is discussed. First, an evaluation of the value of using random regression TD models to analyze milk and milk components data for management purposes will be given. Second, the use of field data in a multi-component analysis
approach to assess cow production response to nutrition will be discussed. Finally, potential applications of research findings described in this thesis are discussed to support technicians as well as farmers in their decision-making strategies.

### 6.2 Management Information system using test-day model

From a production perspective, the dairy sector today is characterized by narrower profit margins than in the past especially for capital-intensive farming systems due to increasing economic pressure: small changes in production or efficiency, therefore, can have a major impact on profitability (Huirne, 1990; van Asseldonk et al., 1999). The adoption of on-farm use of management information systems has been proven to increase herd average production and return on investments (Tomaszewski et al., 2000). Realized benefits from computer use are anticipated to be higher for larger (>300 cows) herds (Lazarus et al., 1990). Furthermore, the progress in information and communication technology in the last decade has made it possible to capture, store and process vast amounts of data from sources on the farm as well as from external organizations. The challenge for dairy producers is to interpret and utilize this information properly to improve decisionmaking.
Data collected in DHI agencies represent the main source of information on farm productivity that can be used to support management decisions. This information is processed and analyzed by DHI agencies to provide farmers with reports that can be used as a basis to make appropriate decisions for the improvement of on-farm management practices. Although DHI data and information can contribute to improved management practices, the benefits are only realized when the farm manager and/or the advisor spend a considerable amount of time in analyzing the information. It is, therefore, necessary to develop analytical tools which will accelerate these analyzes. Such tools would filter and pre-process the data, and would present them under a form and in a way which would predispose them to the analytical process.
In this study a model that exploits test day information (TD model) for management purposes was developed for Sicilian dairy herds. Everett et al. (1994) suggested using results of TD models for monitoring genetics and management in dairy cattle. TD models have been further improved and today represent one of the most advanced and sophisticated mathematical tools to process DHI data with very high reliability. Test-day models are used in most countries to perform genetic evaluations for dairy cattle by using test-day observations instead of aggregated

305-d yield observations (Ptak and Schaeffer, 1993; Reents et al., 1995; Jamrozik et al., 1997a; Schaeffer et al., 2000). By modeling the shape of the lactation curve and the variability of yields around general shapes, TD models provide 4 to $8 \%$ more accurate genetic evaluations of cows compared to evaluations from 305-d yields (Schaeffer et al., 2000). For management purposes, several solutions based on TD models have been proposed in the literature also. As an example, estimation of fixed, genetic, environmental and herd effects can be used to predict future productions of individual cows. Deviations between predicted and actual production could be used to detect a disease at an early stage, i.e. before the cow shows clinical signs. Mayeres et al. (2004) and Pool and Meuwissen (1999) investigated the ability of a TD model to predict yield from TD records. Halasa et al. (2009) used the difference between actual and predicted production to model production loss due to subclinical mastitis. Records from cows with clinical mastitis were excluded in order to use predicted production based only on healthy cows. A multiple-trait mixture model was successfully applied to TD milk yield, fat-toprotein ratio and somatic cell score to detect sub-clinical mastitis in dairy cattle (Jamrozik and Schaeffer, 2012). Fat-to-protein ratio, easily available, highly heritable and relatively independent from milk and somatic cell score, could serve as an additional indicator for indirect selection against mastitis in dairy cattle. The above applications refer to tools to support management decisions at the level of the individual cow. However, TD models can also be used to determine timedependent herd effects, such as herd-test-day or herd-lactation curves effects, which can be used to support management decision at herd level. The herd-testday effect accounts for month-to-month variability and is particularly informative with regard to short-term management changes that affect the whole herd at a particular TD such as a change in feed ration. Koivula et al. (2007) developed a dairy herd management Web application "Maitoisa" (in English, "Milky") to help farmers to recognize systematic patterns and single unusual test days, based on the analysis of monthly herd-management effect solutions from a TD model in Finland. Monthly herd-management effect is defined as a deviation from the mean within each herd. The herd-management effect is especially informative for immediate management changes that affect the whole herd at a precise TD.
In this study we focused on using herd curves (HCUR) from a random regression model to develop management parameters. The difference between our models and management parameters developed in other studies is that we focused on herd curves rather than individual cow information.

From a management perspective, those herd specific lactation curves give information on how a herd of animals performed compared to how they would
have done under average management circumstances. The HCUR variances for milk production traits were highest around peak yield (DIM 50 to 150; Chapter 2). Higher variability at the peak indicates that differences in management between herds are expected to have the largest impact around the peak of the lactation. The ratio of HCUR over phenotypic variance was highest for protein yield around the time of peak yield, with values greater than 1 for first and second lactation, showing that variability between herds is bigger than between animals. For this reason, we decided to develop management parameters for milk, fat, and protein yield at between-herd level rather than at cow level, or rather than within-herds, for example among herd test days.

The high variation in HCUR indicates a promising opportunity to improve management of herds, which should lead to a reduction of variation in HCUR. To explain HCUR variability found in this study, an experiment was performed where routinely information was collected on management practices and nutrition at herd level (every 3 months) and information was collected on production and health status at cow level (every month) on 40 farms in Ragusa province from March 2006 through December 2007. In chapter 3 yearly HCUR traits (peak, mean and persistency) were associated to animal breed and feeding system (separate feeding vs. TMR) variables and to TMR chemical composition. Results from this analysis demonstrated that breed, feeding management, and crude protein and dry matter content in the diet and their interaction influenced significantly HCUR, especially peak, mean, and persistency. In general, HCUR of Holstein Friesian farms had higher milk peak but were less persistent for all traits compared to Brown Swiss farms. TMR fed cows produced on average more milk, fat, and protein, and their curves had a higher peak compared to animals fed with separate feeding. Looking at TMR chemical composition, a significant impact ( $\mathrm{P}<0.05$ ) of crude protein on peak and mean HCUR for all production traits was found. The interaction crude protein $\times$ dry matter had a significant effect ( $\mathrm{P}<0.01$ ) on persistency for all traits and parities, except for fat and protein for first parity cows, whereas neutral detergent fiber $\times$ Starch marginally affected ( $\mathrm{P}<0.1$ ) persistency for milk and protein HCUR in parity 2 and 3 . These results illustrate the power of random regression TD models to process TD milk production data for management advice. Apart from simply processing that data, TD model also allow proper correction for breed and feeding system. The analyses revealed that herd curves parameters derived from the data were highly variable across herds, and the variability turned out to be associated to nutritional management of the farm. These results can be used to advice farmers about (in-)appropriate nutrition: adequate CP content in the diet, as an example.

### 6.3 Multi-component analysis of field data to assess milk production response to dietary changes

### 6.3.1 Data analysis for nutrition experiments

In Chapter 3, the production response to dietary chemical and physical changes was estimated using a retrospective analysis of field data. In literature, dairy cow experiments to assess nutrition effects can be classified in two main categories: continuous and change-over trials. In continuous trials, a cow, once placed on the experimental diet, remains on that diet during the whole trial. In change-over trials, a cow receives two or more treatments during the course of the trial. In a metaanalysis comparing feed intake and milk production responses in continuous vs. change-over design dairy cow experiments, Huhtanen and Hetta (2012) showed that in continuous trials, the high variability between cows often did not allow to detect economically important differences with a realistic number of cows per treatment. In change-over trials, residual errors were smaller compared with the continuous trials, since the variation between cows was excluded from the residual variance. Another advantage of using change-over design was that with a given number of cows more treatments can be designed into the trial. However, the disadvantage of using such experimental design was represented by the so called 'carry-over' or residual effect, and a possible bias in estimating experimental error (Lucas, 1960). Furthermore, the short-term production responses may not reflect the whole lactation responses (e.g. Morris, 1999). Results showed that when the expected differences between the diets in feed intake were large, production responses could be underestimated in change-over designs. Under these circumstances continuous trials may result in more accurate estimates of the differences between the diets, although at the expense of reduced precision. However, when the expected differences between the diets were from small to moderate, change-over designs were more precise and economical requiring less animals, and most likely, as accurate as continuous experiments also for estimating long-term effects.
In this thesis, a different approach to determine the impact of diet composition was used. Existing data were used to explain in retrospect the variation in the lactation curves due to diet composition. A disadvantage of our approach is that the nutritional components were only examined four times a year. However, an advantage was that a large number of herds and animals were included in the analysis. The large dataset enabled correction for genetic and herd-year-season
effects. We were also able to investigate many nutritional components simultaneously by quantifying the substitution effect on production, i.e. the change in production due to increasing/decreasing of one diet component when other components were fixed at three dietary concentrations, under the assumption that animals fed free choice eat to their maximal capacity. However, to handle field data particular attention has to be paid to statistical analysis. TMR were sampled every 3 months, whereas production data are collected on a monthly basis. To associate TMR chemical composition to lactation curves, each TD record was associated to the closest TMR analysis fed to animals immediately before or after the TD. A random regression sire - maternal grandsire model was used to assess this association. To examine the influence of TMR, model fitting included TMR chemical and physical composition, in a one-component analysis, modeled as an interaction with DIM using a ninth-order Legendre polynomial to increase the sensitivity to dietary effects across DIM. These effects were fitted as fixed regressions, to ensure that the random herd test date effect only accounts for the residual that is left after the main effect of main composition. Based on the results of the onecomponent analysis, a multi-component analysis was performed where crude protein, neutral detergent fiber, and starch and their interaction with DIM (fitted as a ninth-order Legendre polynomial) were simultaneously included in the model.

The models presented in Chapter 4 and 5 allowed a retrospective analysis to explain variation in the lactation curves due to diet composition. Selection of animals in homogeneous groups for known variables affecting milk and milk components production (breed, DIM, age at calving, stage of pregnancy) was not necessary, as these variables were included in the TD model as fixed effects. Adjusting for these effects is necessary to avoid misinterpretation of results due to partial confounded by other variables different from the ones under investigation. Also, adaptation period was not needed as cows at time of testing were already under the experimental diet regime. In feeding trials, adaptation time (10 to 14 days recommended, Cochran and Gaylean, 1994) is required as rumen microbes need to adapt to the new diet under evaluation to reach the full and unbiased responses in milk and milk component production. The relatively easy and low-cost access to a large amount of data was, however, counterbalanced by the necessity of collecting TMR for a long period (3 years) on a large number of farms (40). TMR were collected for each farm every 3 months and chemical composition was associated to the closest TD milk test. Data collection did not take into account diet changes and time when a component of the diet was replaced (hay, silage, concentrate), and, as an example, this did not allow the cows to adapt to the new diet changes when and if they occurred a few days before the TMR was collected.

However, the TD model accounts for the correction for this effect, as it quite presumably reflects in the HTD effect, but further investigations are needed to verify this hypothesis.

Although there are obvious caveats to this retrospective analysis of nutritional effects, for example, when only limited data are available, other (unknown) management effects might be confounded with the nutritional parameters and lead to spurious associations, Also, caution has to be paid when interpreting results from this study, as this model was developed based on variation in diets found in Sicily. Association within this environment may not work for other regions, e.g. Northern Italy or even Northern Europe due to differences in the diets found in these regions. Diets in Sicilian herds are characterized by high fiber content of forages due to subtropical climate (Van Soest 1994); different forage sources (limited use of corn silage because of restricted water availability); use of specific by-products such as citrus pulp and carob. Hence, despite these cautions, the results demonstrate that these are out weighted by the advantage of using data from practical circumstances investigating the associations with many nutritional components simultaneously. Several expensive experiments would have been needed to investigate all interactions between components.

### 6.3.2 Effects of nutrition on lactation curves

Based on results found in the literature, greater impact of energy (starch) and forage quality (lignin and acid and neutral detergent fiber) was expected on herd curve traits (Hristov et al., 2002). One reason for this discrepancy could be that energy in the diet affects milk production at cow level: examination of individual animal curves or deviations of real from expected production estimated from the model is thus needed.

To this purpose, data collected at 27 farms located in Ragusa province (Sicily, Italy) in a field study from 2006 through 2008 were analyzed, using a modeling approach, to assess the effects of crude protein (CP), neutral detergent fiber (NDF) and starch (Chapter 4) and particle size (Chapter 5) on individual lactation curves for milk, fat and protein. Two analyses were performed to assess these effects: a onecomponent analysis (NutUni), where each chemical and physical parameter was included in the model one by one; a multi-component analysis (NutMulti), where the main chemical component effects where included simultaneously in the model. Results of responses in this study suggest that changes in milk yield to CP and NDF were dependent on dietary content of starch. Dietary CP was the second most important factor. However, the response to CP was strongly dependent in starch
and NDF content of the TMR as well. Responses resulting from the one-component analysis confirmed the literature. However, more specifically, although increasing starch had a significant impact on milk production throughout lactation in both analyses, a more pronounced effect was found in the NutMulti model than in the NutUni model for starch. Brun-Lafleur et al. (2010) found similar results when assessing the effect of energy $\times$ protein interaction on milk yield: this interaction resulted in a sharper response of milk yield to energy supply for high levels than for low levels of CP supply. When CP is at an average concentration in the diet, increasing starch had a slightly larger impact on increasing peak yield than increasing CP when starch was at an average concentration.

Despite the difference in methodology, the effects of nutritional components on lactation curves found in this study were consistent with reported literature which lends credibility to the multiple component models. The change of production effects when the other nutrients are included in the model found in this study suggest the confounding response one can have when multiple nutrients are not accounted for.
Looking at the effect of physical characteristics of TMR on lactation curves, only little effect of class of particle proportions on the top two sieves of Penn State Particle Separator and peNDF on milk yield and fat yield was found (Chapter 5). Of interest is the effect of sieve classes on protein yield. In this data, protein yields were enhanced when the upper sieve contained 10.4 to $17.4 \%$ particles irrespective of the middle sieve proportions (dietary CP was $15.5 \%$, starch was $19.7 \%$ and NDF was $40.7 \%$ for the analysis). This result was surprising. Only Kononoff and Heinrichs (2003a, 2003b) have observed an influence of particle size on protein content in milk. Most studies examining particle size have not found an effect on milk protein yield or content (Krause, 2002; Krause and Combs, 2003; Yang and Beauchemin, 2005; Bhandari et al., 2007; Bhandari et al., 2008). The distribution most associated with increased protein yield was when the top screen contained $10.4 \%$ to $17.4 \%$ of TMR particles and the lower screen and pan contained $45 \%$ to $59 \%$ of TMR particles. Optimal distributions were different than those recommended and may reflect regional differences based on types of forages fed.

### 6.4 Towards implementation

We have developed a TD model that allows estimation of herd curves and herd curves parameters. Development of management parameters at herd and cow
level is necessary to provide farmers with tools to interpret production data and support them in their decision making task. In this thesis, management parameters focused on herd level development, as variance components of production data collected in Ragusa province, estimated with TD models, showed highest variation in herd curves. Sources of variation of herd curves were identified by breed, feeding system and chemical composition of the diet. Herd curves are therefore a useful tool that can be used to detect the management problem at herd level. However, a proper management information system (MIS) that supports farmers in translating the detection of a problem from herd curves with abnormal patterns into planning of activities (from strategic to operational through tactical) is still missing.
A MIS, according to Davis and Olson (1985), is an integrated, user-machine system for providing information to support operations, management, and decision making functions in an organization and two derivations of MIS have been developed: decision support systems, that support the decision maker to retrieve data and test alternative solutions during the process of problem solving (Devir et al., 1993); or expert systems, that use expert knowledge to attain high levels of performance in a narrow problem area (Hogeveen et al, 1991; Pellerin et al., 1994; Schmisseur and Gamroth, 1993).

As a further evolution of the data processing system presented in this thesis, here we aim to develop a combination of both kinds of MIS, where the random regression TD model is used to retrieve the data and estimate the curves and the results of the management and nutritional associations are used to train the knowledge systems after validation by experts. These results can be used to expand the current information system of CoRFiLaC into a full MIS.
The first step to expand the current system of Corfilac taken was by providing dairy farmers with overviews of their herd curve parameters (figures 6.1 and 6.2) through a restricted-area web-based service.


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## HERD MANAGEMENT $\quad$ C URVES



Figure 6.1 - Herd curves for first, second, and third parity for milk, protein, and fat yield, and somatic cell score associated to "bad management"

## Herd no.: 08700455; Report type: MRM; Date: 01/10/2008

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Figure 6.2 - Herd curves for first, second, and third parity for milk, protein, and fat yield, and somatic cell score associated to "good management"

The herd curves of these 2 farms clearly show differences in production level as well as in curve shapes for all traits. Farm 08710440 (A, Figure 6.1) has a negative production level, around 4 kg milk, 200 g fat, and 150 g protein yield below the average of the population average (Ragusa province in its context). The somatic cell
score for this herd is positive, which means a higher content of somatic cell count compared to the average. Farm 08700455 (B, Figure 6.2), on the contrary, shows a positive deviation for all traits: around 5 kg milk, 110 g fat, and 150 g protein yield higher than the baseline. Somatic cell score deviation is accordingly negative. In both herds, first-parity cows produce less milk, fat, and protein yield, compared to multiparous cows, but are more persistent. Looking at the management characteristics of these 2 herds, farm A has 25 Brown Swiss cows in lactation, housed in tie stall and fed with separate feeding system, whereas farm B has 30 Holstein lactating cows, housed in free stalls and fed with traditional separate feeding system as well. Differences in housing systems may have influenced production at herd level (Simensen et al., 2010). The 2 herds adopted the same feeding systems, however after evaluation of the diets through CPM Dairy (version 3.0.8, University of Pennsylvania, Kennett Square, PA, Cornell University, Ithaca, NY and Miner Agricultural Research Institute, Chazy, NY), also diets adopted by the 2 herds are substantially different. Estimated average ( $\pm$ st dev) CP levels from 1-year diets are 14.1 ( $\pm 1.66$ ) vs. $16.2( \pm 0.31)$ for farm A and B, respectively. As starch and NDF are correlated nutrition parameters in the diets, estimated average starch levels are 15.7 ( $\pm 3.02$ ) vs. $24.8( \pm 1.24)$ for farm A and B, respectively, and NDF levels are $40.5( \pm 2.25)$ vs. $34.2( \pm 0.33)$ for farm A and B, respectively. Diet composition might have the most important effect on lactation herd curves, however caution has to be paid in interpreting such results. Known factors, such as breed, influencing HCUR should be included in the model in order to reduce potential score of variation.
There are examples of MIS in the literature aimed to help farmers managing their herds. A knowledge-based system developed for dairy managers to automatically download through a Direct Access to Records by Telephone (DART) program, inspect and interpret lactation curves for his herd relative to an average of comparable herds in the DHIA, to diagnose potential problems in their herd, and to recommend appropriate strategies for amelioration (Whittaker et al., 1989). Also, a case-acquisition and decision-support system was developed to support the analysis of group-average lactation curves and to acquire example cases from domain specialists (Pietersma et al. 2001). The system, at first step, consisted of interpretation rules derived from scientific literature written by nutritionists and extension specialists. The knowledge base was then submitted to the attention of dairy consultants to refine and enhance the justifications and recommendations of the rules. This set could be expanded by a set of prototype rules consisting of causal dependencies, justifications and recommendations. For example, the nutritional associations found in Chapter 3 could be used to instruct the system.

Also, further investigations of other management parameters (e.g., housing systems, diets evaluation) that may affect production curves at herd level are needed. The rules derived in the learning phase are then used to interpret abnormal deviations of herd average production from the population base. However, knowledge acquisition through interviews has proven to be timeconsuming and difficult. Experts often have difficulty expressing how they make their decisions and, in addition, it is not easy to structure and encode the knowledge expressed through interviews into a representation that can be used as part of a KBS. Alternatively, knowledge acquisition can be partially automated with machine learning (Langley and Simon, 1995; Dhar and Stein, 1997). With this approach, a domain expert first classifies example cases of a particular problem. A machine-learning technique, such as decision-tree induction, is then used to learn how to classify new cases based on these examples. In our case, the system would be trained using the equations resulting from the associations found in this thesis between breed, feeding systems and chemical and physical characteristics of the diets with lactation curves, combined with validation performed by experts in management and nutrition.

## REFERENCES

Brun-Lafleur, L., L. Delaby, F. Husson, and P. Faverdin. 2010. Predicting energy $\times$ protein interaction on milk yield and milk composition in dairy cows. J. Dairy Sci. 93:4128-4143.
Cochran, R.C., Galyean, M.L., 1994. Measurement of in vivo forage digestion by ruminants. In: Fahey, G.C., Collins, M., Mertens, D.R., Lowell, L.E. (Eds.), Forage Quality, Evaluaiton, and Utilization. American Society of Agronomy, Madison, USA, pp. 613-639.
Davis, G. B. and Olson, M. H. 1985. Management information systems. Conceptual foundations, structure, and development. New York: McGraw-Hill.
Devir, S., J. A. Renkema, R. B. M. Huirne, A. H. Ipema. 1993. A new dairy control and management system in the automatic milking farm; basic concepts and components. J. Dairy Sci. 76:3607-3616.
Everett, R. W., F. Schmidt, and L. H. Wadell. 1994. A test-day model for monitoring management and genetics in dairy cattle. J. Dairy Sci. 77(Suppl. 1):267. (Abstr.)
Halasa, T., M. Nielen, A.P.W. De Roos, R. Van Hoorne, G. de Jong, T.J.G.M. Lam, T. van Werven, and H. Hogeveen. 2009. Prduction loss due to new subclinical mastitis in Dutch dairy cows estimated with a test-day model. J. Dairy Sci. 92:599606.

Hogeveen, H., E. N. Noordhuizen-Stassen, J. F. Schreinemakers, A. Brand. 1991. Development of an integrated knowledge-based system for management support on dairy farms. J. Dairy Sci. 74:4377-4384.
Hristov, A. N., W. J. Price, and B. Shafii. 2002. Dietary factors influencing milk yield and milk protein yield in dairy cows. Pages 147-166 in Proc. Pacific Northwest Animal Nutr. Conf., Boise, ID.
Huirne, R. 1990. Basic concepts of computerized support for farm management decisions. Euro. R. Agr. Eco. 17:69-84.
Huhtanen, P., M. Hetta. 2012. Comparison of feed intake and milk production responses in continuous and change-over design dairy cow experiments. Livest. Sci. 143: 184-194.
Jamrozik, J., L. R. Schaeffer, and J. C. M. Dekkers. 1997. Genetic Evaluation of Dairy Cattle Using Test Day Yields and Random Regression Model. J. Dairy Sci. 80:12171226.

Jamrozik, J. and Schaeffer L. R. 2012. Test-day somatic cell score, fat-to-protein ratio and milk yield as indicator traits for sub-clinical mastitis in dairy cattle. J. Anim. Breed. Genet. 129:11-19.
Koivula, M., J. I. Nousiainen, J. Nousiainen, and E. A. Mäntysaari. 2007. Use of herd solutions from a random regression test-day model for diagnostic dairy herd management. J. Dairy Sci. 90:2563-2568.
Kononoff, P. J., and A. J. Heinrichs. 2003a. The effect of corn silage particle size and cottonseed hulls on cows in early lactation. J. Dairy Sci. 86:2438-2451.
Kononoff, P. J., and A. J. Heinrichs. 2003b. The effect of reducing alfalfa haylage particle size on cows in early lactation. J. Dairy Sci. 86:1445-1457.
Lazarus, W. F., D. Streeter, and E. Jofre-Giraudo. 1990. Management information systems: impact on dairy farm profitability. North Cent. J. Agric. Econ. 12(2):267277.

Lucas, H.L., 1960. Critical features of good dairy feeding experiments. J. Dairy Sci. 43: 193-212.
Mayeres, P., J. Stoll, J. Bormann, R. Reents, and N. Gengler. 2004. Prediction of daily milk, fat, and protein production by a random regression test-day model. J. Dairy Sci. 87: 1925-1933.
Morris, T.R., 1999. Experimental Design and Analysis in Animal Sciences. CABI Publishing, Oxon, UK. 208 p.
Pellerin, D., R. Levallois, G. St-Laurent, J. -P. Perrier. 1994. LAIT-XPERT VACHES: an expert system for dairy herd management. J. Dairy Sci. 77:2308-2317.

Pietersma, D., R. Lacroix, D. Lefebvre, E. Block, and K. M. Wade. 2001. A caseacquisition and decision-support system for the analysis of group-average lactation curves. J. Dairy Sci. 84:730-739.
Pool, M. H., and T. H. E. Meuwissen. 1999. Prediction of daily milk yields from a limited number of test days using test day models. J. Dairy Sci. 82: 1555-1564.
Ptak, E., and L. R. Schaeffer. 1993.Use of test day yields for genetic evaluation of dairy sires and cows. Livest. Prod. Sci. 34: 23-34.
Reents, R., J. C. M. Dekkers, and L.R. Schaeffer. 1995. Genetic evaluation for somatic cell score with a test day model for multiple lactations. J. Dairy Sci. 78:2858.

Schaeffer, L. R., J. Jamrozik, G. J. Kistemaker, and B. J. Van Doormaal. 2000. Experience with a Test-Day Model. J. Dairy Sci. 83:1135-1144.

Schmisseur, E., M. J. Gamroth. 1993. DXMAS: an expert system program providing management advice to dairy operators. J. Dairy Sci. 76:2039-2049.
Simensen, E., O. Østerås, K. E. Bøe, C. Kielland, L. E. Ruud, G. Næss. 2010. Housing system and herd size interactions in Norwegian dairy herds; associations with performance and disease incidence. Acta Veterinaria Scandinavica 2010, 52:14.
Tomaszewski, M. A., A. A. Dijkhuizen, R. B.M. Huirne, A. Otten. 2000. Management information system impact on dairy production for selected herds in Texas. Comp. and Electronics in Agric. 26: 1-11.
van Asseldonk, M.A.P.M., R.B.M. Huirne, A.A. Dijkhuizen, A.J.M. Beulens, A.J. Udink ten Cate. 1999. Information needs and information technology on dairy farms. Comp. and Electronics in Agric. 22: 97-107.
Van Soest, P. J. 1994. Nutritional ecology of the ruminant. Cornell University Press, Ithaca, New York.
Whittaker, A. D., M. A. Tomaszewski, J. F. Taylor, R. Fourdraine, C. J. van Overveld, R. G. Schepers. 1989. Dairy herd nutritional analysis using knowledge systems techniques. Agr. Syst. 31: 83-96.

## Summary

## Samenvatting

## Acknowledgements

## Publications

Training and supervision plan
Curriculum vitae

Colophon

## Summary

Management information is crucial both for accurate monitoring and for adequate planning of activities at modern dairy farms. Milk recording is recognized as a valuable tool for herd management world-wide, and milk recording provides an important source of information for estimation of breeding values. Analyses of data for management and for genetic evaluation have long been separate processes with different statistical procedures and frequencies of data processing. Random regression models that use test-day records for milk yield have been implemented for the estimation of breeding values. Recent studies have investigated the possibility to use a test-day model for management purposes. The aim of this study was to investigate the use of test-day information to support farmers in management of Sicilian dairy herds.
The specific objectives of this thesis were 1) to develop the test-day random regression model for the analysis of production data of Sicilian dairy herds, 2) to develop parameters from the random regression model that that can be used to advise dairy farmers on nutritional management of their dairy cows, and 3) to investigate the production response to changes in chemical and physical composition of diets in Ragusa province (Sicily, Italy). For objective 2 to 3 a field study was conducted in Southern Italy (Ragusa province) where diet and chemical composition of the diet was collected at herd level (every 3 months) and testday milk yield records at individual cow level (every month). Data collection was performed from March 2006 through December 2008 on 40 cooperating farms.

First, benefits in using a random regression TD model for management improvement (Chapter 2) were investigated. Variance components for milk, fat, and protein yield, and somatic cell score of dairy cows in Ragusa province were estimated. Higher variance of the lactation curves at herd level (between herds) compared to the phenotypic variance (between animals) around lactation peak were found, suggesting that development of management parameters for milk, fat, and protein yield around the peak should focus on herd level rather than cow level parameters, to evaluate and improve management between herds in Ragusa province.

Sources of variation that explain herd curves were explored in Chapter 3. Random herd curves for milk, fat, and protein yields were estimated from a random regression test-day model per herd, year, and parity (1, 2, and 3+) using 4 -order Legendre polynomials. Traits describing herd curves (peak, days in milk at peak, mean, and persistency) were associated to breed of animals (Holstein Friesian vs. Brown Swiss), feeding system (total mixed ration vs. traditional separate feeding) and total-mixed-ration chemical composition (dry matter, ash, crude protein, soluble nitrogen, acid detergent lignin, neutral detergent
fiber, acid detergent fiber, and starch). Feeding system affected significantly herd curve peak and mean. Brown Swiss herds showed significantly higher persistency, whereas Holstein Friesian showed higher herd curve peak and mean. Crude protein had the largest effect on herd curve peak and mean, whereas the interaction between crude protein and dry matter mainly affected persistency. Herd curves can therefore be used as an indicator of herd feeding management.

Based on results found in the literature, greater impact of energy and forage quality on herd curve traits was expected. The response in milk and milk components production to varying diet chemical composition was then investigated at individual cow level (Chapter 4). The analyses were performed by combining individual lactation curves, estimated using a random regression sire-maternal grandsire test-day model, with chemical composition of total mixed rations fed to animals. Results showed that starch had the greatest effect on milk, fat, and protein production when crude protein and neutral detergent fiber contents were at a high and low value, respectively. The change in production effect when the other nutrients are included in the model found in this study suggests the confounding response one can have when multiple nutrients are not accounted for.

In Chapter 5 the association of total-mixed-ration physical properties with production of milk, fat, and protein was assessed. Total-mixed-ration physical property was estimated as particle fractions retained on the Penn State Particle Separator. Particle size distribution in Ragusa dairy farm total mixed rations were associated only with small but significant effects on milk protein yield. Optimal distributions were different than those recommended and may reflect regional differences based on types of forages fed.

In Chapters 4 and 5, a different approach compared to most experiments investigating production responses to varying nutritional quality was developed: the field study approach allowed a retrospective explanation of lactation curves variation due to diet chemical and physical composition on a very large dataset including a large number of herds and animals.

Finally, in the general discussion presented in chapter 6, the contribution of the analyses performed in this thesis to development of management parameters derived from milk recording system information are discussed. First, the value of using random regression test-day models to analyze milk and milk components data for management purposes was evaluated. Second, the use of field data in a multi-component analysis approach to assess cow production response to nutrition was discussed. Finally, potential applications of research findings described in this thesis to support technicians as well as farmers in
their decision-making strategies were discussed. A proper management information system that supports farmers in translating the detection of a problem from herd curves into consequent planning of activities can be set up using the parameters estimated in this thesis.

## Samenvatting (Summary in Dutch)

Management informatie is cruciaal zowel voor nauwkeurige monitoring als voor een adequate planning van de activiteiten op moderne melkveebedrijven. Melkproductieergistratie wordt wereldwijd erkend als een waardevol instrument voor bedrijfsmanagement. Daarnaast zijn melkproductiegegevens een belangrijke bron voor het schatten van fokwaarden. Analyses van gegevens voor deze twee doeleinden zijn al lange tijd gescheiden processen met verschillende statistische procedures en verschillende frequentie van de gegevensverwerking. Random regressiemodellen die gebruik maken van proefmelkgegevens, de zogenaamde testdagmodellen, zijn reeds geïmplementeerd voor het schatten van fokwaarden. Recente studies hebben de mogelijkheid van het gebruik van het testdagmodel voor management doeleinden onderzocht. Het doel van dit onderzoek was om de bruikbaarheid van proefmelkgegevens ter ondersteuning van de veehoudersin het mangement van Siciliaanse melkveebedrijven te onderzoeken.

De specifieke doelstellingen van dit proefschrift waren 1) ontwikkelen van testdag random regressie model voor de analyse van de melkproductiegegevens van Siciliaanse melkveebedrijven, 2) ontwikkelen van parameters uit het random regressie model die gebruikt kunnen worden om melkveehouders te adviseren over voeding van hun melkkoeien, en 3) het effect van chemische en fysische samenstelling van de voeding te onderzoeken op melkproductiecurves. Voor doelstelling 2 tot 3 werd veldonderzoek uitgevoerd in Zuid-Italië (provincie Ragusa) waar naast de routine proefmelkgegevens, ook het rantsoen en chemische samenstelling van het rantsoen werd verzameld. Het verzamelen van gegevens werd uitgevoerd van maart 2006 tot en met december 2008 betreffende de 40 samenwerkende bedrijven.

Ten eerste werd het gebruik van een random regressie testdagmodel onderzocht (Hoofdstuk 2). Variantiecomponenten voor melk-, vet- en eiwitproductie en celgetal van melkkoeien in provincie Ragusa werden geschat. Hogere variantie van de lactatiecurven werd gevonden tussen bedrijven, in vergelijking met de fenotypische variantie (tussen dieren) rond de piekproductie tijdens de lactatie. Dit suggereerde dat de ontwikkeling van managementparameters voor melk-, vet- en eiwitproductie rondom de lactatiepiek zich moeten richten op bedrijfsniveau in plaats van koeniveau , om het management te verbeteren in de provincie Ragusa.

Bronnen van variatie die verschillen in bedrijfscurves verklaren werden onderzocht in Hoofdstuk 3. Bedrijfscurves voor melk-, vet- en eiwitproductie werden geschat op basis van een random regressie testdagmodel. Eigenschappen dei de curves beschrijven (piek, dagen in melk tijdens de piek,
gemiddelde productie, en persistentie) waren geassocieerd met ras (Holstein Friesian vs Brown Swiss), voersysteem (totaal gemengd rantsoen vs traditionele aparte voeding) en de samenstelling van het TMR rantsoen (droge stof, as, ruw eiwit, verteerbare stikstof, lignine, vezels en zetmeel). Voersysteem had een significant effect op de piekproductie en gemiddelde productie. Brown Swiss bedrijven toonden significant hogere persistentie, terwijl bedrijven met Holstein Friesians een hogere piek en een hoger gemiddelde hadden. Ruw eiwit had de grootste invloed op de hoogte van de bedrijfscurve en het gemiddelde van de bedrijfscurve, terwijl de interactie tussen ruw eiwit en droge stof de persistentie beïnvloedde. Verschillen in bedrijfscurven kunnen daarom gebruikt worden als indicator voor wijze van voeren.

Op basis van de resultaten gevonden in de literatuur, werd een grotere impact van de energie- en ruwvoerkwaliteit op de melkproductiecurves verwacht. Daarom werd onderzocht hoe de chemische samenstelling van het rantsoen effect had op individueel koeniveau (Hoofdstuk 4). De analyses werden uitgevoerd door het schatten van individuele lactatiecurves, en deze te combineren met het effect van de chemische samenstelling van het rantsoen. De resultaten toonden aan dat zetmeel het grootste effect had op melk-, veten eiwitproductie wanneer ruw eiwit en neutraalbestande vesel inhoud een hoge en lage waarde hadden, respectievelijk. De verandering in het effect op productie, nadat de andere nutrienten in het model meegenomen waren, geeft aan dat je een verstrengeld effect kunt hebben wanneer geen rekening wordt gehouden met meerdere nutrienten.

In hoofdstuk 5 is gekeken naar de invloed van de deeltjesgrootteverdeling van het TMR op de melkproductiecurves. Deze werden bepaald met de Penn State Particle Separator. Deeltjesgrootteverdeling op Ragusa melkveebedrijven was alleen geassocieerd met een klein maar significant effect op eiwitproductie. Optimale verdeling van de fracties lag anders dan aanbevolen op basis van resultaten uit ander landen. Dit kan verklaard worden door verschillen in de soorten gevoerde ruwvoeders .

De multicomponent analyse van praktijkdata met behulp van random regressiemodellen, zoals in hoofdstuk 4 en 5, voegt een extra benadering toe bij het onderzoek naar de effecten van voeding op productie. In vergelijking met de dominerende experimentele aanpak kan via deze benadering achteraf verklaard worden hoe verscheidene chemische en fysische componenten van het rantsoen (en de interacties) een effect hebben op de lactatiecurve van melkvee, onder praktijkomstandigheden en met een relatieve grote dataset met een groot aantal bedrijven en dieren.

Ten slotte, in de algemene discussie in hoofdstuk 6, is de toegevoegde waarde van random regressiemodellen voor management informatie bediscussieerd, en het gebruik van praktijkgegevens in een multi-component analyse om voedingseffecten te schatten. Tot slot is beschreven hoe de resultaten uit dit proefschrift gebruikt kunnen worden bij de ondersteuning van voorlichters en boeren ter ondersteuning van hun besluitvorming. Een goed management informatiesysteem dat veehouders ondersteunt bij het vertalen van de gevonden afwijkingen van productiebedrijfscurves naar consequente planning van werkzaamheden, kan worden opgezet op basis van de parameters geschat int dit onderzoek.

## Acknowledgements

This thesis was intended to continue the important research that was carried out by Prof. Licitra, pioneer in Sicily in supporting dairy farmers and leading to the foundation of CoRFiLaC. I thank him for having made this dream possible and for having trusted and supported me.

Prof. Roel Veerkamp and prof. Johan van Arendonk allowed me to study in one of the most important agricultural universities in the world. Thanks Johan for your kindness, availability, and always encouraging words. Thanks Roel for your tenacity, resolution, and rigour: I have learned a lot from your professionalism, accuracy, and leading capacity.

A very special thanks goes to prof. Jim Ferguson: he has always been present during the whole PhD project and acted as the father I no longer have. Thanks also to prof. David Galligan for his precious suggestions on data collection.

I'm grateful to Gerben de Jong and Sander de Roos at NRS in Arnhem, and Marco Pool and Wijbrand Ouweltjes at the Animal Sciences Group in Lelystad for having introduced me to the world of "test-day models". Their involvement was very important to set up this research.

The local milk recording agency (APA) in Ragusa, and all cooperating farms are acknowledged for having provided the data necessary to this study. Alberto Cappa is particularly acknowledged for having provided production data of Vicenza province and for his contribution in interpreting outputs from test-day models.

Thanks to John Hickey and Sebastian Mucha who introduced me to the "Dutch life-style". Special thanks also to my friends Patrizia Buldo and loanna Stampelou who never made me feel far from home.

Many thanks to the very efficient Animal Breeding and Genomics Center Secretariat, and especially to Lisette Bourquin for her precious help in finalizing the thesis and organizing all the bureaucracy issues while staying in Sicily.

The support of CoRFiLaC and of my colleagues was crucial to carry out the project: thank you to all of you. In particular, I want to thank Mariano Gambina, Carmelo Scollo, Emiliano Gurrieri, Gaetano Fichera, Alfio Pappalardo, Mario Dipasquale, and Gabriele Valesio. They fulfilled the hardest part of the project by collecting data and samples. My special thanks go to Saro Petriglieri and Giuseppe Azzaro who also coordinated all the on-farm activity and had always encouraging words that helped me throughout the whole project. Salvatore

Ventura, Alessio Pozzebon, and Giuseppe Giurdanella are gratefully acknowledged for their help in data editing, program running, and data analyses. I would like to thank Mario Manenti, Stefania La Terra, Maria Ottaviano, Giovanna Campisi, Carmelo Guardiano, Giovanni Longombardo, Stefania Cilia, Michela Minà, Giovanni Farina, Glenda Leto, and Lucia Cascone for their contribution with chemical analyses. Special thanks go to Francesca La Terra and Iris Schadt for their help with for editing and interpreting results, and also to Vincenzo Guastella for having designed the cover of this thesis. I'm grateful to Catia Pasta, Ivana Piccitto, Stefania Carpino, Giuseppe Azzaro, Saro Petriglieri and all other friends at CoRFiLaC: you've always been there, ready to support me in any difficulty.

I want to express my gratitude from the bottom of my heart to my beloved family: my husband Marcello, my daughter Elena and our long-awaited unborn Sofia. They have shared with me, most of all others, sacrifices, successes, and sorrows. I dedicate this achievement to them.

Warmest thanks to my mother, my mother-in-law, my sister, my brother and his family, my sister- and brother-in-law and their families, for having helped me in managing routine life while studying and working.

Finally, I would like to dedicate this thesis to my Dad Nino who was looking forward to seeing me getting my PhD. Although you are no longer with us, I am sure you are proud of me.

## LIST OF PUBLICATIONS

## Peer-reviewed publications

Caccamo, M., R.F. Veerkamp, G. Licitra, R. Petriglieri, F. La Terra, A. Pozzebon, and J.D. Ferguson. 2012. Association of total-mixed-ration chemical composition with milk, fat, and protein yield lactation curves at individual level. J. Dairy Sci. 95: 6171-6183.

Caccamo, M., R.F. Veerkamp, J.D. Ferguson, R. Petriglieri, F. La Terra, and G. Licitra. 2010. Association of breed and feeding management, and tmr composition with herd curves from random regression test-day model. J. Dairy Sci. 93: 4986-4995.

Caccamo, M., R.F. Veerkamp, G. de Jong, M.H. Pool, R. Petriglieri, and G. Licitra. 2008. Variance components for test-day milk, fat and protein yield, and somatic cell scores for analyzing management information. J Dairy Sci. 91: 3268-3276.

Caccamo, M., C. Melilli, D.M. Barbano, G. Portelli, G. Marino, and G. Licitra. 2004. Measurement of gas holes and mechanical openness in cheese by image analysis. J. Dairy Sci. 87:739-748.

## Co-authored publications

Fucà, N., C. Pasta, G. Impoco, M. Caccamo, G. Licitra. 2012. Microstructural properties of milk fat globules. International Dairy Journal. http://dx.doi.org/10.1016/j.idairyj.2012.03.010.

Schadt, I., J.D. Ferguson, G. Azzaro, R. Petriglieri, M. Caccamo, P. Van Soest , and G. Licitra. 2012. How do dairy cows chew? —Particle size analysis of selected feeds with different particle length distributions and of respective ingested bolus particles. J. Dairy Sci. 95:4707-4720.

Impoco, G., N. Fucà, C. Pasta, M. Caccamo, G. Licitra. 2012. Measuring shape and distribution of nanostructures in micrographs using image analysis. Computers and Electronics in Agriculture 84, 26-35.

Fucà, N., D.J. McMahon, M. Caccamo, L. Tuminello, S. La Terra, M. Manenti, and G. Licitra. 2012. Effect of brine composition and brining temperature on cheese physical properties in Ragusano cheese. J. Dairy Sci. 95:460-470.

Ben Younes, R., M. Ayadi, T. Najar, M. Caccamo, I. Schadt, M. Ben M’Rad. 2011. Effect of thermal stress, cistern size and milking frequency on plasma mineral concentrations in Holstein dairy cows. J Life Sciences. 5(2011) 739746.

Impoco, G., L. Tuminello, N. Fucà, M. Caccamo, G. Licitra. 2011. Segmentation of structural features in cheese micrographs using pixel statistics. Computers and Electronics in Agriculture, 79(2), 199-206.

Ben Younes, R., M. Ayadi, T. Najar, M. Caccamo, I. Schadt, M. Ben M’Rad. 2011. Hormonal (Thyroxin, Cortisol) and immunological (Leucocytes) responses to cistern size and heat stress in Tunisia. J Life Sciences. 5 (2011) 257-265.

Azzaro, G., M. Caccamo, J.D. Ferguson, S. Battiato, G.M. Farinella, G. C. Guarnera, G. Puglisi, R. Petriglieri, and G. Licitra. 2011. Objective estimation of body condition score by modeling cow body shape from digital images. J. Dairy Sci. 34:2126-2137.

Melilli, C., D.M. Barbano, M. Caccamo, L. Tuminello, S. Carpino, and G. Licitra. 2006. Interaction of brine concentration, brine temperature, and presalting on salt penetration in Ragusano cheese. J. Dairy Sci. 89: 1420-1438.

Melilli, C., D.M. Barbano, M. Caccamo, M.A. Calvo, G. Schembari, and G. Licitra. 2004. Influence of brine concentration, brine temperature, and presalting on early gas defects in raw pasta filata cheese. J. Dairy Sci. 87: 3648-3657.

## Other publications and abstracts

Caccamo, M., A. Pozzebon, G. Azzaro, R. Petriglieri, G. Licitra. 2010. Curve di lattazione a livello aziendale: un indicatore dell'effetto delle pratiche manageriali sulla produzione e la qualità del latte. Poster presentato a Torino il 23 Settembre 2010 al II $^{\circ}$ Congresso Lattiero-Caseario

Caccamo, M., R.F. Veerkamp, G. Licitra, R. Petriglieri, F. La Terra, A. Pozzebon, and J.D. Ferguson. 2012. Multi-component versus one-component analysis: a different way of assessing the effect of TMR chemical composition on milk, fat, and protein yield individual lactation curves. July 14-19, Phoenix, Arizona, USA. J. Anim. Sci. Vol. 90, Suppl. 3/J. Dairy Sci. Vol. 95, Suppl. 2, 248.

Caccamo, M., G. Azzaro, G. Gallo, G.C. Guarnera, J.D. Ferguson, G. Licitra. 2011. Automatic estimation of body condition score from digital images. X, American Dairy Science Association. July 10-14, New Orleans, Louisiana, USA.

Caccamo, M., J.D. Ferguson, R.F. Veerkamp, I. Schadt, R. Petriglieri, G. Azzaro, A. Pozzebon, G. Licitra. 2011. Effect of total mixed rations particle fractions retained on the Penn State Particle Separator on milk yield lactation curves using a random regression animal model. X, American Dairy Science Association. July 10-14, New Orleans, Louisiana, USA.

Caccamo, M., R.F. Veerkamp, R. Petriglieri, F. La Terra, and G. Licitra. 2010. Effect of TMR chemical composition on milk yield lactation curves using a random regression animal model. Abstract. EAAP meeting, August 23rd-27th, Heraklion, Crete, Greece.

Azzaro, G., M. Caccamo, J.D. Ferguson, S. Battiato, G.M. Farinella, G.C. Guarnera, G. Puglisi, G. Licitra. 2009. Modeling Cow Body Shape for Objective Estimation of Body Condition Score from Digital Images. Montreal. ADSA meeting.

Caccamo, M., R.F. Veerkamp, J.D. Ferguson, R. Petriglieri, F. La Terra, G. Licitra. 2009. Influence of Feed Management on Random Herd Curves from Random Regression Test-Day Model. Montreal. ADSA meeting.

Caccamo, M., G. Impoco, L. Tuminello, and G. Licitra. 2008. Three-dimensional microscopy using stereoscopy applied to scanning electron microscopy imagery. Abstract. Indianapolis, Indiana. American Dairy Science Association.

Caccamo, M., R.F. Veerkamp, G. de Jong, M.H. Pool, R. Petriglieri, G. Azzaro, G. Licitra. 2007. Variance components of test-day milk, milk components, and somatic cell score useful for management advice. S. Antonio, Texas. American Dairy Science Association.

## Publications

Caccamo, M., G. Impoco, F. Zanini, G. Tromba, P. Campo, S. Carpino, G. Licitra. 2007. New alternative approaches to study cheese microstructure. S. Antonio, Texas. American Dairy Science Association.

Caccamo, M., G Impoco, L Tuminello, and G Licitra. 2006. Quantitative analysis of cheese microstructure by scanning electron microscope images. Minneapolis, Minnesota. American Dairy Science Association.

Licitra, G., M. Caccamo, I. Schadt, J.D.Ferguson, G. Gennuso and G. Azzaro. 2005. Method to measure feed particles by image analysis. OHIO. American Dairy Science Association.
Training and supervision plan
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Presentations (5 ECTS)"Variance of test-day milk, milk components, and somatic cell score2007useful for management advice", ADSA-PSA-AMPA-ASAS Joint AnnualMeeting, San Antonio, Texas (USA), July 9, oral"Variance of milk, milk components, and somatic cell score useful2007
for management advice", Nutrition and Management of DairyCattle, Ragusa, June 5-9, oral"Influence of Feed Management on Random Herd Curves from2009Random Regression Test-Day Model ", ADSA-CSAS-ASAS JointAnnual Meeting, Montreal, Quebec (Canada), July 16, oral"Effect of TMR chemical composition on milk yield lactation curves2010using a random regression animal model", EAAP 61st Annual
Meeting, Heraklion, Crete Island (Greece), August 25, oral"Multi-component vs one-component analysis: a different way of2012assessing the effect of TMR chemical composition on milk, fat, andprotein yield individual lactation curves", ADSA-AMPA-ASAS-CSAS-WSASAS Joint Annual Meeting, Phoenix, Arizona (USA), July 16, oral
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## Curriculum vitae

## Curriculum vitae

Margherita Caccamo was born on November 4, 1977 in Modica, Ragusa province, Italy. After graduating from high school in modern languages in 1996, she studied Computer Science in Catania University and graduated in 2002 with a thesis on "Body Condition Score and Fuzzy Logic". From 2001 through 2004 she worked at CoRFiLaC as programmer and web editor, and after 2004 through 2009 as researcher and head of the data processing center of CoRFiLaC. In 2005, she leaded a project on "Development of management parameters using a random regression test-day model". In 2006 she started a PhD program at Wageningen University that resulted in this thesis. Data collection and analysis necessary for the PhD study were carried out in Ragusa, whereas she completed her academic training in Wageningen. From 2009, she is the scientific coordinator and responsible of institutional research activity of CoRFiLaC.

## Colophon

The research described in this thesis was carried out at CoRFiLaC (Ragusa, Italy) and financially supported by CoRFiLaC and the Assessorato Agricoltura e Foreste della Regione Siciliana (Palermo, Italy).

The printing of this thesis was funded by the Animal Breeding and Genomics Centre, Wageningen University, Wageningen, the Netherlands.

This thesis was printed by GVO printers \& designers B.V., Ede, the Netherlands.


[^0]:    ${ }^{a, b}$ Means per parity within a trait for feeding system or breed not sharing the same superscript differ significantly ( $\mathrm{P}<0.05$ ).
    ${ }^{1}$ Means were estimated for feeding system and animal breed groups.
    ${ }^{2}$ The effect of the interaction between animal breed and feeding system was significant.
    ${ }^{3}$ Persistency was estimated as $P=\frac{1}{245} \sum_{i=61}^{305} y_{i}-y_{60}$ (Kistemaker, 2003), where $y_{i}=$ yield at DIM $i$.

