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1. THE SELECTION INDEX AND OTHER COMPOSITE INDEXES

1.1. PFT (Productivity, Functionality and Type)

PFT is the selection index of the Italian Holstein breed; it combines milk quality and functionality. The official ranking is based on PFT and ranges from 0 to 99 dividing the population in percentiles. The ranking is calculated separately for bulls and cows. The 99 Rank identifies the best 1% of bulls and cows with calculated breeding values. Since December 2011, Rank 98 (best 2% of the population) is the official limit for a bull to be used in A.I.. The table below shows the relative weight of each trait included in PFT.

The formula for PFT is as follows:

\[
PFT = 12.50 \times (0.32 \times \text{fat kg} + 1.79 \times \text{protein kg} + 0.087 \\
\times \text{fat\%} \times 100 + 0.28 \times \text{protein\%} \times 100 + 4.04 \times \text{type} + 13.93 \\
\times \text{ICM} + 6.07 \times \text{IAP} + 9.21 \times ((\text{longevity} - 100)/5) + 11.51 \\
\times ((\text{somatic cell score} - 100)/5.7) + 10.59 \times ((\text{fertility} - 100)/5))
\]

which determines a 49:51 ratio between production and functionality.

<table>
<thead>
<tr>
<th>PRODUCTION</th>
<th>WEIGHTS</th>
<th>FUNCTIONALITY</th>
<th>WEIGHTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk</td>
<td>0</td>
<td>TYPE</td>
<td>4</td>
</tr>
<tr>
<td>Fat</td>
<td>8</td>
<td>ICM</td>
<td>13</td>
</tr>
<tr>
<td>Protein</td>
<td>36</td>
<td>Feet&amp;Legs (IAP)</td>
<td>6</td>
</tr>
<tr>
<td>Fat %</td>
<td>2</td>
<td>Somatic cells</td>
<td>10</td>
</tr>
<tr>
<td>Protein %</td>
<td>3</td>
<td>Functional longevity</td>
<td>8</td>
</tr>
</tbody>
</table>


1.2. ICM (Udder Composite Index)

The goal of this index is to breed for a functional udder. Its formula is based on percentages of weights relative to the linear scores of the udder, defined according to their relation to the functional longevity of the animals and is as follows:

\[
\text{ICM} = 0.19 \times \text{fore attachment strength} + \\
0.17 \times \text{rear attachment height} + \\
0.21 \times \text{ligament} + \\
0.26 \times \text{udder floor} + \\
0.17 \times \text{teats placement}
\]

\[
\text{ICM} = 0.19 \times (\text{fore attachment score} - 100)/10 + 0.17 \times (\text{rear attachment score} - 100)/10 + 0.21 \times (\text{ligament score} - 100)/10 + 0.26 \times (\text{udder floor score} - 100)/10 + 0.17 \times (\text{teats placement score} - 100)/10
\]
1.3. IAP (Feet & Legs Composite Index)

Used for the first time in May 2000, this index combines three linear traits relative to feet and legs and locomotion (expressed by the subjective evaluation of the classifier). The relating weights express (as a percentage) the relative importance of each trait (due to correlations) in relation to the trait ‘feet & legs functionality’, and are the following:

\[
IAP = 0.160 \times \text{foot angle} + \\
0.244 \times \text{rear leg rear view} + \\
0.408 \times \text{locomotion} + \\
-0.208 \times \text{rear leg set}
\]

1.4. TYPE

From October 2014 index for TYPE is derived from 17 linear traits to ensure the same definition across all bulls Italian and foreign and young and older. The formula has been derived from genetic correlation among the different traits and the genetic correlations with the actual definition of Final Score officially used by classifiers. The relative weight of each trait is as follows:

\[
\text{TYPE} = 0.000 \times \text{stature} + 0.128 \times \text{strength} + 0.031 \times \text{body depth} + \\
0.158 \times \text{angularity} + 0.044 \times \text{rump angle} + \\
0.013 \times \text{rump width} - 0.025 \times \text{rear legs side view} + \\
0.038 \times \text{rear legs rear view} + 0.050 \times \text{foot angle} + \\
0.175 \times \text{udder front} + 0.072 \times \text{rear height} + \\
0.030 \times \text{udder support} + \\
0.014 \times \text{udder depth} + 0.050 \times \text{front teat} - \\
0.023 \times \text{teat size} - 0.032 \times \text{rear teat} + 0.016 \times \text{locomotion}
\]
2. BREEDING VALUES FOR PRODUCTION TRAITS AND SOMATIC CELL SCORE (SCS)

2.1. Data recording

The Italian Breeders Association (AIA) receives every month all the milk records collected by the personnel of the Provincial Associations (APAs). Alternate recording data (AT) are projected to 24 hours records complying ICAR standards. All available records are used. Every lactation has a weighting factor expressing its accuracy:
- AT records are worth 98% of monthly complete records (A records).
All the records collected between day 5 and day 305 of lactation are used, up to third lactation. Besides ordinary data editing, records abnormally deviating from each cow’s estimated lactation curve are discarded.

2.2. Environmental effects

A random regression test day model is adopted, estimating every effect using a function that explains their variability from day 5 to day 305 of lactation. Breeding values for milk, fat, protein and somatic cells are estimated simultaneously, thus making use of genetic correlations among traits that improve total accuracy of estimates. Estimated fixed effects are age at calving, calving season (spring or autumn), area of production (Northern, Central or Southern Italy and “Parmesan cheese area”) and the five-years period (“quinquennium”) in which production occurred (from 1990). Average variability of milk, fat and protein within herds are calculated every year, using all available test-day records; this parameter measures variability of herd productions around total average and helps in identifying higher and lower than average variability herds before adjusting them to match the “correct” scale. The “correct” scale is within-herd variability of primiparous cows born between 2007 and 2009 (the genetic base cows). This correction for heterogeneity of variance allows differences among animals to be measured in a unique scale for every herd. Animals are compared according to herd-year-test day and parity. Cows are divided into first- and multiple parity class; this last effect allows to account for every management effect within herd and time.

2.3. Random effects

The animal effect, after which the Animal Model statistical method is named, allows for the estimation of breeding values of all animals within the population. In the random regression test day model the hypothesis is that animals express a different genetic value each day of lactation in response to environmental stimulation. Besides, all animals with (cows) or without (bulls) production records are considered simultaneously, together with all their relationships, allowing for the deviation from contemporaries to be resolved into its dam and sire components and taking into account, for instance, the fact that on the best cows usually the best available bulls are used. A second
Random effect is the permanent environmental effect: each cow can have more than one lactation. Therefore it is necessary to estimate occasional management effects that may have a negative (a mastitis which severely damages one of the quarters), or a positive (a very positive interaction with an healthy environment) influence on her lifetime production. Also in this case, a 5 parameters function is estimated.

2.4. How the breeding value is expressed

The traits for which breeding values are calculated are milk, fat and protein yields and somatic cell score (SCS).

2.4 Production traits

Heritability varies according to parity and day of lactation and usually is around 0.30, thus establishing a 30:70 ratio between genetic and environmental variability.

The three resulting breeding values, for first, second and third lactation, are then combined in an index of total three-lactation production:

$$EBV_{305_{tot}} = 0.333 \times EBV_{305_1} + 0.333 \times EBV_{305_2} + 0.333 \times EBV_{305_3}$$

This index is expressed in kilograms as deviation from a reference base, the genetic base, that sets the zero of breeding values. The genetic base is a rolling base and it is updated in April every year; its purpose is to express the breeding values relative to the value of the cows in the herds at the present time. Current genetic base reflects genetic value of the cows born between 2007 and 2009 and has been update in April 2015. Every year the triennium will be moved forward one year.

Percentages are computed with reference to the 305 days phenotypic productions of cows of the genetic base. The base values are then used in the following formula to calculate the breeding values for percentages:

$$\text{fat\%} = 100 \times \frac{(\text{fat base} + \text{fat})}{(\text{milk base} + \text{milk})} - \text{fat\% base}$$
$$\text{protein\%} = 100 \times \frac{(\text{protein base} + \text{protein})}{(\text{milk base} + \text{milk})} - \text{protein\% base}$$

The base values are published on the web page in the section related to genetic proofs (http://www.ANAFI.it).

A bull is officially proven when he reaches a minimum reliability of 80% and has at least 30 daughters with 120 DIM. For all foreign bulls are used the results provided by INTERBULL (decision n° 256 of the Central Technical Committee 25/10/2013).
2.5 Somatic cell score

Heritability varies according to parity and day of lactation and usually ranges from 0.17 in first lactation to 0.25 in third lactation. The resulting three breeding values for first, second and third lactation are combined in a total index for SCS, that express the mean somatic cell score in the three lactations, using the following formula:

\[ \text{EBV}_{\text{tot}} = 0.333 \times \text{EBV}_1 + 0.333 \times \text{EBV}_2 + 0.333 \times \text{EBV}_3 \]

This index is expressed on a scale with mean equal to 100 and standard deviation equal to 5. Current genetic base represents the genetic value of cows born between 2005 and 2007 as for the production traits. Also for SCS indices, the Italian proof is the official one if reliability is at least 75% with daughters in 20 or more herds; INTERBULL proofs are used otherwise.

2.6 Persistency index and maturity rate

The individual lactation curves that the random regression test-day model provides for each cow, allow us to quantify the genetic component for persistency of production; to express it with an indicator, we calculate the percent ratio between 280 days and 60 days productions. The index is on a scale with mean 100 and SD equal to 5. There are three indexes for persistency, one for each lactation, and a combined indicator computed using the following formula:

\[ \text{PERS}_{\text{tot}} = 0.50 \times \text{PERS}_1 + 0.25 \times \text{PERS}_2 + 0.25 \times \text{PERS}_3 \]

Maturity rate measures, on the other hand, the difference between the sum of fat and protein kilograms in third and first lactation. The results is then expressed on a scale with mean 100 and SD equal to 5. It tells us which are the bulls whose daughters improve performance through lactations (values above 100), compared to those whose daughters perform the best in first lactation (values below 100).
3. BREEDING VALUES FOR TYPE TRAITS

3.1. Data recording

Information of type evaluations carried out on all first-calf heifers classified two or four times a year by the breed inspectors (over the last ten years) is used for the calculation of the breeding values. Unlike to what happens with the production proofs, in this case age variation is not so strong and there is no repeated data. For each cow only one evaluation is used.

3.2. Environmental effects

Among the animals classified in a herd there are differences by age and stages of lactation which undoubtedly influence their body condition and appearance. All traits are evaluated together in a multiple traits analysis. The inspector measures what he/she sees, therefore the statistical model must take into consideration this different condition by using an interaction effect between age and stage of lactation by two year period.

Cows are compared among them on an equal herd-year-round of classification: this allows to simultaneously take into account all managerial effects of each single herd and also the year and the classifier who evaluated the animal.

3.3. Random effects

The only random effect taken into consideration is that of the animal. All parental relationships existing among the population are taken into consideration, and this allows to estimate the genetic value of all animals, taking into account possible preferential or corrective mating.

3.4. How the breeding value is expressed

The breeding values of all linear type traits evaluated since 1984 and final score are calculated. Table illustrates the heritability data utilized for the calculation. The breeding value for teat size has been introduced in 1994 and in May 2000 a composite breeding value for rear legs (rear view) and feet and legs functionality have been added.

A zero breeding value refers to the genetic level of animals born between 2007 and 2009, i.e. the genetic base, for which the same rules of the production traits apply. All breeding values are standardized for the variability of the cows in the genetic base. In this way, all traits can be measured on a same scale which goes from –3 to +3 both for cows and bulls. In reality, bulls and cows do not vary in the same way, and therefore bulls that have high breeding values, especially for production traits, being all highly selected and well above the cows average, tend to vary towards extremes which are higher than 3. On the Table you can see the heritability values of all traits, the phenotypic average of the animals in the genetic base, and the equivalent in points (linear scale) of one standard deviation of that breeding value. The table values
will vary at every change of the base and will be updated on the web page where genetic proofs are.
The breeding value is published when a bull has at least 10 daughters in 5 herds, in case of bulls with daughters in Italy. Conversion formulae of breeding values to the Italian scale are used for bulls imported from abroad with daughters still not classified. For foreign bulls the official proof is the Italian one of INTERBULL.

3.5 New Locomotion Index

Locomotion evaluates the length of the step and direction of rear legs in movement and is scored on a 1 (lame) to 50 (excellent) scale, with 1-unit increments: a score of 5 is given to short strides and severe abduction; a score of 25 is given to normal strides and slight abduction; and scores greater than 40 are given to long strides without abduction. The diseases of feet and legs are expensive to routinely detect, making it difficult to create an archive suitable for the genetic evaluation of animals. Locomotion, detected during the morphological evaluations can be used as an indicator for the pathologies.

Validated in September 2014, locomotion is included in Feet & Legs Composite Index and TYPE index.

The heritability for locomotion is equal to 4%, indicating that the environmental component has a significant effect in the variability of this trait. The genetic correlations between locomotion and arts viewed from the side is good (0.62) and also with feet & legs functionality (0.61). The correlation with heel height is 0.53 and with limbs viewed from behind is -0.26. The correlations between these traits suggest that select animals with a high genetic value for locomotion could have a positive effect on traits relative to feet and legs.

Genetic base 2007-2009

<table>
<thead>
<tr>
<th>Linear Trait</th>
<th>Heritability</th>
<th>Average Base</th>
<th>SD in linear points</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stature</td>
<td>0.40</td>
<td>32.12</td>
<td>2.50</td>
</tr>
<tr>
<td>Strength and vigor</td>
<td>0.25</td>
<td>27.90</td>
<td>1.52</td>
</tr>
<tr>
<td>Body Depth</td>
<td>0.30</td>
<td>30.93</td>
<td>1.69</td>
</tr>
<tr>
<td>Angularity</td>
<td>0.23</td>
<td>28.20</td>
<td>1.32</td>
</tr>
<tr>
<td>Rump Angle</td>
<td>0.23</td>
<td>25.20</td>
<td>1.59</td>
</tr>
<tr>
<td>Rump width</td>
<td>0.22</td>
<td>26.70</td>
<td>1.61</td>
</tr>
<tr>
<td>Rear leg set</td>
<td>0.16</td>
<td>26.19</td>
<td>0.98</td>
</tr>
<tr>
<td>Heel depth</td>
<td>0.10</td>
<td>24.90</td>
<td>0.83</td>
</tr>
<tr>
<td>Fore attachment strength</td>
<td>0.20</td>
<td>24.24</td>
<td>1.59</td>
</tr>
<tr>
<td>Rear attachment height</td>
<td>0.21</td>
<td>27.06</td>
<td>1.43</td>
</tr>
<tr>
<td>Rear attachment width</td>
<td>0.24</td>
<td>29.76</td>
<td>1.15</td>
</tr>
<tr>
<td>Ligament</td>
<td>0.16</td>
<td>29.25</td>
<td>1.19</td>
</tr>
<tr>
<td>Udder floor</td>
<td>0.30</td>
<td>29.01</td>
<td>2.33</td>
</tr>
<tr>
<td>Teats front placement</td>
<td>0.19</td>
<td>24.66</td>
<td>1.18</td>
</tr>
<tr>
<td>Teats size</td>
<td>0.19</td>
<td>22.59</td>
<td>1.18</td>
</tr>
<tr>
<td>Rear leg rear view</td>
<td>0.06</td>
<td>25.38</td>
<td>0.72</td>
</tr>
<tr>
<td>Feet and legs functionality</td>
<td>0.10</td>
<td>22.95</td>
<td>0.85</td>
</tr>
<tr>
<td>Rear Teat Placement</td>
<td>0.18</td>
<td>27.60</td>
<td>1.79</td>
</tr>
<tr>
<td>Conformation</td>
<td>0.14</td>
<td>25.56</td>
<td>1.41</td>
</tr>
<tr>
<td>Locomotion</td>
<td>0.04</td>
<td>21.54</td>
<td>0.50</td>
</tr>
</tbody>
</table>
4. BODY CONDITION SCORE (BCS)

4.1. Data recording

Body condition score is a visual measure of fat covering the pelvic and lumbar regions; its scoring is based on a 1 (very thin) to 5 (very fat) scale with 0.25-point increments. In particular, the fat reserves of the thurl region, the angularity of hips and pins, and the prominence of spinous processes are evaluated two or four times a year by breed inspectors. This information is used for calculation of breeding values. The index was considered in the official national evaluation from December 2013.

4.2. Environmental effects

The production changes over time and so does the animal situation in the farm. There are time dependent factors such as the age effects on the lactations stage. Animals are compared according to herd-year-test day and parity. Cows are divided into first- and multiple parity class; this last effect allows to account for every management effect within herd and time.

4.3. Random effects

The only random effect taken into consideration is that of the animal. All parental relationships existing among the population are taken into consideration, and this allows to estimate the genetic value of all animals, taking into account possible preferential or corrective mating.

4.4. How the breeding value is expressed

The results is then expressed on a scale with mean 100 and SD equal to 5. It tells us which are the bulls whose daughters are more conformed (values above 100) with a better body condition. Each standard deviation (which is equal to a 5 on the scale of the index) is worth about 0.08 points BCS: bulls with a genetic level of less than 90 have daughters with BCS equal to 2.78, bulls with EBV between 95 and 105 have daughters with BCS of between 2.94 and 3.01 and bulls with indexes above 110 have daughters with BCS equal to 3.16 points.

<table>
<thead>
<tr>
<th>Genetic bulls level</th>
<th>Phenotopic average of daughter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Below to 90</td>
<td>2.78</td>
</tr>
<tr>
<td>From 90 to 94</td>
<td>2.85</td>
</tr>
<tr>
<td>From 95 to 109</td>
<td>2.94</td>
</tr>
<tr>
<td>From 100 to 104</td>
<td>3.02</td>
</tr>
<tr>
<td>From 105 to 109</td>
<td>3.09</td>
</tr>
<tr>
<td>Above 110</td>
<td>3.16</td>
</tr>
</tbody>
</table>
5. BREEDING VALUE FOR CALVING EASE

5.1. Data recording

During milk testing, the technicians of the Provincial Association also gather all available information on as many events as possible: births, deaths, sales, breeding, calving, etc. Data on calving include the dairy farmer evaluation of the degree of difficulty, on a five categories scale:

A = easy calving
B = help needed from one person only
C = cesarean
D = difficult calving
E = embryotomy

Each category is evaluated on a difficulty scale going from 0 to 100, where easy calving is 0 and embryotomy is 100. The sex of the new born calf is also used to further differentiate the various degrees of difficulty.

Data used for present evaluation were collected starting from 1987. From August 2003 we discard herd-year classes that:
- had more than 92% records coded as A;
- had more than 92% records coded as B;
- had more than 15% records coded as C and over.

5.2. Environmental effects

In the model the interaction between year and month of calving, the interaction between province and year and the interaction among the age of the dam, the sex of the new born and the parity of the cow are taken into account.

5.3. Random effects

The effects of herd-year, the sire and the maternal grandsire are considered as random.

5.4. How the breeding value is expressed

There are two resulting breeding values: one is the direct effect of the bull (sire of the calf) and the second one is the maternal effect or the calving ease of the daughters. Both are on a scale with mean 100 and SD of 5 units. Bulls with values above 100 have an higher percentage of easy calving. Heritability is near 10%, which shows that for this trait there is a strong predominance of the environmental effects in determining what is really going to happen at calving. There is no genetic base to refer to since there is no selection for this trait.
6. BREEDING VALUE FOR MILKABILITY

6.1. Data recording

Twice a year, the persons in charge of the milk recording collect also information on the milking speed of the cows. More specifically, they ask the milkers to point out the cows which are below average for milking speed. Repeated reports about the same cow will determine her degree of slowness: the cow that is always identified as below average in milking speed at each survey, will have a score of 1; the cow surveyed three times and identified as “slow” one time only, will have a score of 0.33, and so on. Her contemporaries are her herdmates of the first survey or of the time when she was first identified as “slow”, if there have been variations in time.

6.2. Environmental effects

The different lactation number of the surveyed cows is taken into account; an adjustment is made also for the amount of milk produced on the test day and for the herd-year-test day. By doing so it is possible: a) to avoid mistaking high yields with slow milking; b) to account for different management practices, in particular for the management of the milking parlor and of the personnel in charge.

6.3. Random effects

Another relevant factor is the effect of the animal, therefore in the evaluation of the genetic value of the bulls it is taken into account the possible slowness of the dam of the surveyed cow.

6.4. How the breeding value is expressed

Only the sires breeding values are published and the heritability of the trait is 6%, due to the limited accuracy of the data collected. The value is expressed as a genetic value (IGT) on a scale with mean 100 and SD of 5. The value 100 corresponds to the value average breeding value of the genetic base (cows born between 2007 and 2009). The average phenotypic value of cows in the base are published on the web site. The phenotypic average is generally around 4%.
7. THE BREEDING VALUE FOR FUNCTIONAL LONGEevity

7.1. Data recording

The general lactations record gives a precious information: the productive career of all the Holsteins under milk recording. It’s known for how many lactations an animal has produced before disappearing from the farm, at what productive levels his productions were located compared to the farm average, how often she due etc. It’s possible to notice, over time, the trend of the farms: some of them are stable, some are at their closing point and other are enlarging themselves. All these data, if properly analysed, tell us the dynamic of the animals elimination from the farm. Next to those animals which have already closed their career, there are those who have just started it.

7.2. Environmental effects

The production changes over time and so does the animal situation in the farm. There are some stable factors which do not change such as the first calving age of an animal, that, by the way, determines his survival capacity over time. There are time dependent factors such as the age effects on the lactations stage, the milk kg. productive level within the year, divided into nine classes (four under the average, average, four above the average), the fat percentage productive level within the year (5 classes), the protein percentage productive level within the year (5 classes), the yearly changes in the herd size, and at the end the year-season effect on which external factors have an influence, among those the recoveries, the market dynamics (quota) or the pathologies that bring to extraordinary elimination patterns of the animals.

7.3. Random effects

The bull, maternal grandsire and year-season effects are considered. The applied model, based on the survival functions, derives from medical studies and is called survival analysis; it is a sire-maternal grandsire model.

7.4. The combined index

The accuracy of the survival index of the bulls, in particular for the young animals, those who have all the daughters still in production, is definitely low, a little bit higher than a pedigree index. Such an index doesn’t tell us much on the real survival of his daughters. But we know that from the type evaluation of the daughters is possible to get a partial information on their longevity. From this originates the composed index for the longevity: survival index, feet & legs and udder index are combined together taking into consideration the genetic correlation between the several characters in order to compute the final combined longevity index. In particular the correlation between udder and functional longevity is 0.48 and the one between legs functionality and longevity is 0.14.
7.5. How the breeding value is expressed

Just the index of those bulls which have at least a reliability of 50% or higher is published. The heritability of this trait is around 10%. The longevity index is expressed like a genetic value (IGT) on a scale with mean 100 and SD of 5 just like for the other functional traits, minimum and maximum value are between 80 and approximately 120.

7.6. WHAT DOES IT REALLY MEAN

The functional longevity concept is not easy to explain: is not the rough longevity the one which is possible to observe in the farm, because this one is correlated to the productive level of the animals. The concept itself of longevity is related to fertility, to health of the animal, etc.

Besides this, between functional longevity and real longevity, and by this we mean the number of lactations that the animal is able to carry on during his career, there is a precise relation.

In order to try to quantify the real difference between animals, carrier of very positive longevity genes, and those carrier of genes with very negative effects on the longevity of their daughters, we computed a simple statistic of the data for the bulls having more than 200 daughters and born between 1980 and 1988.

The differences we found are listed in the below table:

<table>
<thead>
<tr>
<th>Genetic level</th>
<th>Average number of daughters’ lactation</th>
<th>Average number of eliminated daughters’ lactation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lower than 90</td>
<td>2.30</td>
<td>2.10</td>
</tr>
<tr>
<td>Around 100</td>
<td>2.58</td>
<td>2.30</td>
</tr>
<tr>
<td>Higher than 110</td>
<td>3.20</td>
<td>2.90</td>
</tr>
</tbody>
</table>

The expected difference is of one lactation. Another very similar result, has been calculated in Canada, where the genetic standard deviation is approximately 1/4 of lactation and therefore the difference between the best and the worst bulls is around one lactation.
8. THE BREEDING VALUE FOR FERTILITY

8.1. Data recording

The data base for inseminations and calving are the information used for genetic evaluation for fertility. On this data base several checks guarantee that only good quality records will be used for genetic evaluation (about 20% of the total records are discarded). The majority of records discarded are questionable first insemination data. Together with this data bases also linear trait and lactation data base are used.

For a complex trait as fertility in fact, direct and indirect traits are used all together. The direct traits considered are: days to first insemination, non return rate at 56 days and calving interval. Indirect traits are: angularity and ME 305 first parity lactation. All the traits are measured on first parity cows in order to evaluate daughter fertility of all bulls.

The five traits are considered simultaneously in order to exploit at best the genetic correlation that do exist across these traits and maximize the estimation precision.

8.2. Environmental effects

Every trait is affected by different and specific environmental factors. Thus every traits has its own statistical model. Factor accounted for days to first inseminations and calving interval are:

a) herd-year-season of calving,
b) calving month,
c) age at calving by year of calving;

for non return rate at 56 days factors are:

a) herd-year-season of insemination,
b) month of insemination,
c) age at calving within year of calving by year of insemination;

environmental factors considered for angularity are the same used for genetic evaluation for type traits:

a) age at calving by stage of lactation,
b) herd-year-round of classification.

Last but not least environmental factor considered for 305ME milk is simply the herd-year-season of calving effect.

8.3. Random effects

The animal effect is evaluated thus considering bull and cows at the same time, accounting for genetic level of mating and using all available pedigree information. It is a multiple trait animal model.
8.4. The combined index

At the end of the computation step five genetic proofs are available. The objective of selection is to improve fertility in general terms. In order to achieve this in an easy to understand and simple way the chosen objective traits is conception rate at first service. This trait is genetically correlated to each of the five evaluated traits and the final proofs combines all of them to maximize the improvement on conception rate giving to each of the relative importance shown in the Table below.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Genetic correlation with conception rate</th>
<th>Relative importance in the combined proof</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days to first insemination</td>
<td>-0.18</td>
<td>16</td>
</tr>
<tr>
<td>NRR56</td>
<td>+0.42</td>
<td>17</td>
</tr>
<tr>
<td>Calving Interval</td>
<td>-0.62</td>
<td>51</td>
</tr>
<tr>
<td>Angularity</td>
<td>-0.15</td>
<td>7</td>
</tr>
<tr>
<td>305ME milk</td>
<td>-0.17</td>
<td>9</td>
</tr>
</tbody>
</table>

8.5. How the breeding value is expressed

Just the index of those bulls which have at least a reliability of 50% or higher is published. The heritability of the traits varies from 2 to 26%. The fertility index is expressed like a genetic value (IGT) on a scale with mean 100 and SD of 5 just like for the other functional traits, minimum and maximum value are between 80 and approximately 120. Bulls with higher fertility have values greater than 100.

8.6. What does it really mean?

In spite of the low heritability value, especially for direct traits, the genetic variability among animals is very high. In the top 5% bull population group for PFT there are individuals that have an 85 proof and other that do reach 110. Every standard deviation (that correspond to a value of 5 on the proof scale) above 100 correspond to: +2.67% conception rate, -8 days calving interval, -2 days to first insemination and +0.68% on NRR56.

The differences we found among bulls in the populations are listed in the table below:

<table>
<thead>
<tr>
<th>Daughter phenotypic average</th>
<th>Bull genetic level</th>
<th>Calving interval</th>
<th>Days to 1st insemination</th>
<th>Non return rate 56 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Below 90</td>
<td>439</td>
<td>93</td>
<td>60%</td>
</tr>
<tr>
<td></td>
<td>Around 100</td>
<td>422</td>
<td>89</td>
<td>63%</td>
</tr>
<tr>
<td></td>
<td>Above 110</td>
<td>405</td>
<td>85</td>
<td>69%</td>
</tr>
</tbody>
</table>
9. INDIRECT GENETIC INDEX FOR MILK COAGULATION PROPERTIES (ITC)

9.1. Data recording

Cheese manufacture is the first destination of milk produced in Italy. The transformation of milk into cheese is a process consisting of several stages. Coagulation is one of the most important stage, in which milk change from liquid status to gel. Commonly, the main milk coagulation traits studied are milk rennet coagulation time (RCT, min) and curd firmness (a30, mm). These traits are recorded using systems based on mechanical methods called lattodinamografia. Despite the importance of cheese processing in Italy and the genetic variability observed, is not available a direct genetic selection for cheese making. This is mainly due to the absence, up to now, of a collection of individual data on a large scale and the difficulty of creating a large database.

For the genetic evaluation of dairy processing from December 2013 indirect traits are used (correlated with RCT and a30). The traits are respectively: percentage of protein, fat percentage, somatic cells and variant of k-casein.

9.2. How the breeding value is expressed

The traits are combined and weighted according to their importance (due to correlations) in predicting the index ITC, calculated in Veneto and cheese yield. The result is an index expressed with mean 100 and standard deviation 5. Bulls with index greater than 100 are considered improvers to dairy processing.

9.3. WHAT DOES IT REALLY MEANS

The index commends bulls with favorable values for the breeding values of protein and fat percentage, somatic cells and the bulls with the genotypes of k-casein AB and BB. The bulls with ITC greater than or equal to 105, have average equal to 0.11 (EBV protein %), 0.23 (EBV fat %) and 102 (EBV somatic cells) and 61% of the bulls have the genotype AB for the k-casein and 13% genotype BB. The bulls ITC with more than 95 have an average of -0.08 breeding values (EBV protein %), -0.21 (EBV fat %), 95 (EBV somatic cells), and 8% of the bulls have the genotype AB for k-casein and only 0.29% have the genotype BB.

Averages of protein, fat and somatic cell EBV in function of ITC level index:

<table>
<thead>
<tr>
<th>EBV</th>
<th>ITC ≤ 95</th>
<th>ITC ≥ 105</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (%)</td>
<td>-0.08</td>
<td>0.11</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>-0.21</td>
<td>0.23</td>
</tr>
<tr>
<td>Somatic Cell</td>
<td>95</td>
<td>102</td>
</tr>
</tbody>
</table>
10. GENOMIC EVALUATION

Since December 2011 genomics (the branch of molecular biology that studies the genome/gene pool of living organisms) will become a new selection tool in the hands of breeders of the Italian Holstein. Until now, the methods used to estimate the genetic value of commercial animals has been based on two types of information:

a. Phenotype data (observable performance of daughters)
b. Pedigree record (on sire, dam and progeny)

With the invention of new DNA analysis technologies it is now possible to “know” a part of an individual’s genes (genotype), estimate its value and calculate a **genomic index**.

This new methodology can be used to:

1. estimate the genetic value of a young animal with greater reliability than a pedigree index;
2. reduce the generation interval;
3. maximize the choice of animals to be sent to progeny tests and/or to be used as sires of bulls/cows;
4. increase the reliability of bulls undergoing testing when the number of daughters is limited.

In short, a genomic index is calculated as follows:

1. genotyping individual animals;
2. collecting phenotypes (traditional genetic indexes - EBV);
3. estimating the value of the individual markers (SNP) which determines the genotype;
4. calculating the direct (DGV) and overall (GEBV) genomic index.

Let’s examine in detail how the new methodology was developed at ANAFI.

10.1. Data

The data used, refer both to the genotypes of the bulls and to their traditional genetic indexes and is used as the baseline phenotype for estimating the effects of individual markers.

The genotypes were collected thanks to ANAFI’s collaboration on two research projects (SelMol and Prozoo), a cooperative agreement with three AI centers in Italy (Ciz, Inseme and Intermizoo), exchanging material with certain European countries (Ireland and Switzerland), and an international agreement with North America and England. More than 50,000 genotypes are now available. The distribution of genotypes by origin can be seen on table 1. These genotypes are for proven sires, young bulls and cows. It must be noted that the number of genotyped animals is continuously increasing, since AI centers are continuing to collect samples from calves so that they can preselect the best animals.

### Number of genotypes collected through the various projects and/or agreements

<table>
<thead>
<tr>
<th>Project/Agreement</th>
<th>Number of Genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>SelMol</td>
<td>1035</td>
</tr>
<tr>
<td>ProZoo</td>
<td>1143</td>
</tr>
<tr>
<td>ELICA (ANAFI, CIZ, INSEME, INTERMIZOO)</td>
<td>3219</td>
</tr>
<tr>
<td>North-America</td>
<td>49257</td>
</tr>
<tr>
<td>Switzerland</td>
<td>75</td>
</tr>
<tr>
<td>Ireland</td>
<td>142</td>
</tr>
<tr>
<td>England</td>
<td>1063</td>
</tr>
</tbody>
</table>
10.2. Statistical model

In order to calculate a genomic index, one must estimate the value of the individual markers (currently around 50,000) which make up the genotype of the individual. This estimate assumes that a mathematical model is used that starts with the traditional indexes of a group of highly reliable (proven) sires and provides estimating equations based on individual markers. These equations can be used to calculate the genomic value of animals that are young or without progeny. The estimation process can be summed up as follows:

1. the input data is cleaned;
2. the reference population is identified (proven sires);
3. the effects of markers are estimated using the traditional EBV values of the proven sires;
4. the genomic index of all the animals (proven sires + young animals) is calculated.

The model used at ANAFI to estimate these effects includes not only the 40,000 markers obtained after initial editing (cleaning) procedures, but also a so-called "polygenic" effect, which is entered in the model through the kinship information of the animals.

Studies that have been performed reveal that individual markers cannot explain the full range of variability which is observed. The missing part of the variability can be “recovered” by incorporating kinships between the animals into the model:

\[
\text{EBV} = \text{Markers} + \text{Classic kinship} + \text{error}
\]

10.3. Genomic index for bulls

A direct genomic index can be calculated both for sires with daughters and for bulls without daughters (young bulls) after the value of the individual markers has been determined.

For sires with daughters, direct genomic information is combined with traditional information to increase reliability. The final result is the GEBV. The weight of the two indexes (direct genomic and traditional EBV) depends on the number of available daughters: as the number increases, the weight of the direct genomic index decreases. This correlation can be observed in figure 1, where the weight of genomics as a function of number of daughters is reported for the GPFT.

**Figure 1. Proven sires**: Contribution of the direct genomic index to the GEBV as a function of number of daughters.
For young bulls, the direct genomic index (DGV) may be combined with the pedigree index (a young bull has no daughters, so no traditional index is available). Actually, the model used already includes the pedigree effect, so if a combination with the DGV is made, a double count of classic kinship information may result. To solve this problem, which has not yet been resolved using calculation methodology, it is useful to employ the genomic index obtained directly from the model without combining it with other information.

10.4. Genomic index for cows

A direct genomic index can be calculated both for cow and heifer. All traits can be calculated with the exception of the calving ease. Direct genomic breeding value (DGV) are estimated on the basis of the markers and pedigree (polygenic effect). For cows (animals with information on their performance), are combined the traditional index (IGV) and those calculated using DNA analysis (DGV). Their traditional index (IGV) is not based only on pedigree but also on their performance (lactations, score, etc.) and therefore has more reliability than pedigree index. The weight of the two indexes (direct genomic and traditional EBV) depends on the number of lactations: as the number increases, the weight of the direct genomic index decrease. This is still lower than what happens with the proven bulls. In fact, the conventional indexes of the cows have less information than the proven bulls, for which the reliability, and therefore the weight is much lower. In practice, while for proven bulls their genomic index is similar to that calculated with the daughters in cows are found major differences between IGV and genomic index.

For young animals, of which there is no available information on their performance or their offspring, the direct genomic index (DGV) may be combined with pedigree index.

<table>
<thead>
<tr>
<th>Indexes</th>
<th>Proven bulls</th>
<th>Haifers/Calves</th>
<th>Cows</th>
</tr>
</thead>
<tbody>
<tr>
<td>Traditional index (IGT/IGV)</td>
<td>Pedigree+daughters</td>
<td>Pedigree</td>
<td>Pedigree+phenotype</td>
</tr>
<tr>
<td>Direct genomic index (DGV)</td>
<td>DNA+pedigree</td>
<td>DNA+pedigree</td>
<td>DNA+pedigree</td>
</tr>
<tr>
<td>Genomic index (GEBV)</td>
<td>DGV &amp; IGT</td>
<td>DGV</td>
<td>DGV &amp; IGT</td>
</tr>
</tbody>
</table>

10.5. Reliability

One of the greatest advantages of genomic evaluation is the superior reliability of the indexes obtained especially when bulls without daughters are considered. At present, a young bull is evaluated only using a pedigree index, whose reliability rarely exceeds 35%. Using genomics, indexes can be obtained with a reliability of approx. 65-70%, which is approximately double the value obtained with the classic method. For cow is the same, genomic index have a reliability around 60-70%. A newborn heifer with DNA analysis has about the same reliability of a cow of three years with a lactation. This sharp increase of reliability, compared with the traditional pedigree that has about 30% of reliability, should push to use more the number of heifers used as potential bull dams.

Observing the national selection index (PFT) in figure 2, one can see the difference in reliability between traditional evaluation and genomic evaluation, both for proven sires (Italian or foreign) and for young bulls.
In young bulls the average increase in reliability for PFT is approx. 25%, but is lower for proven sires. This result is to be expected because it shows that genomics adds little for sires with daughters, since the data for estimating them is provided by their daughters.

10.6 Genetic trend

Trend of GPFT in recent years has had a considerable increase; in the graph of gPFT it is easily seen that the increase of gPFT 2009-2013 is significantly higher than that of the pre-genomic era. If before the average increase / year was about 160 points gPFT, from 2009 onwards, the average annual increase exceeds 350 points.

Figure 3. Genetic Trend gPFT: Bulls born in the pre-genomic era (2003-2008) are represented by the symbol of the triangle, while the bulls born after the introduction of genomics (2009-2013) are presented with the circle.
The stability of proven sires. An important aspect for proven sires is the stability of their index’s between successive runs. This can be analyzed by comparing the trend over time of the average differences between the genomic indexes of sires obtained between successive runs. (Figures 4 and 5).

**Figure 4. A comparison of the average differences in genomic index of protein.** Average from August 2014/December 2014, by date of run of genomic index of protein (d.gebvp)

![Protein Variation: mean GEBVs](image)

**Figure 5. A comparison of the average differences in genomic udder composite index.** Average from August 2014/December 2014, by date of run of genomic index of udder composite index (d.gebvicm)

![Udder Composite Index Variation: mean GEBVs](image)

Theoretically the differences should hover around 0, with greater variability in the more recent runs. This is indicated in both figure 4 and figure 5. The genomic index does not introduce lesser stability.
11. THE INTERNATIONAL PROOFS

11.1 Data

Input data for International genetic evaluation are the Official Breeding Values computed in each Country participating to the service. The service of international evaluation is run by International Bull Evaluation Service (INTERBULL) in Sweden. All Countries, including Italy, are sending their official proofs to this centre at a given date determined by a calendar of publication approved each year. All breeding values for bulls with at least 10 daughters in 10 herds, are sent to INTERBULL. For test day model data the proof sent to INTERBULL is the proofs for first, second and third lactation weighted according to number of test days. In April 2015 the traits for which an international evaluation was provided were: milk, fat, protein, standard conformation traits, calving ease direct and maternal, somatic cells, fertility and body condition score.

11.2 MACE MODEL

Each trait is evaluated by INTERBULL with a Multiple Across Country Evaluation (MACE in brief): in practice the protein proof in each Country contributing to the evaluation is considered as a separate trait. In this way is possible to compare bull rankings across countries. The difference across countries is measured through genetic correlation among the various Countries. The factors accounted for in the model are the Country average and the genetic groups for the maternal grand dam and for the material grand sire in case they are born before 1986. Table 1 shows the genetic correlations between Italy and some of the participating Countries for all the traits for which the international evaluation is provided.

<table>
<thead>
<tr>
<th>Trait</th>
<th>USA</th>
<th>CAN</th>
<th>FRA</th>
<th>DFS</th>
<th>NLD</th>
<th>DEU</th>
<th>AUS</th>
<th>NZL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein Type</td>
<td>0.91</td>
<td>0.89</td>
<td>0.87</td>
<td>0.89</td>
<td>0.85</td>
<td>0.86</td>
<td>0.75</td>
<td>0.75</td>
</tr>
<tr>
<td>Somatic cells</td>
<td>0.91</td>
<td>0.91</td>
<td>0.93</td>
<td>0.93</td>
<td>0.88</td>
<td>0.94</td>
<td>0.81</td>
<td>0.76</td>
</tr>
<tr>
<td>Longevity</td>
<td>0.72</td>
<td>0.78</td>
<td>0.69</td>
<td>0.61</td>
<td>0.47</td>
<td>0.76</td>
<td>0.43</td>
<td>0.30</td>
</tr>
<tr>
<td>Fertility</td>
<td>0.93</td>
<td>0.71</td>
<td>0.62</td>
<td>0.85</td>
<td>0.83</td>
<td>0.82</td>
<td>0.85</td>
<td>0.65</td>
</tr>
<tr>
<td>Calving ease</td>
<td>0.60</td>
<td>0.66</td>
<td>0.68</td>
<td>0.75</td>
<td>0.71</td>
<td>0.65</td>
<td>0.59</td>
<td>-</td>
</tr>
</tbody>
</table>

1 – DFS = Denmark, Finland, Sweden.
11.3 How it works

When a bull has a proof only in one Country the model transfers, using the available information about genetic correlation and difference in scale in another Country, the daughter deviation i.e. the bull proof once we subtract from it the parent average. The final proof of the bull in the other Country is then computed adding to the rescaled deviation the parent average in the new Country. When a bull has daughters in more than one country the deviations are weighted by the number of daughters and then summed together before re-adding the pedigree in the Country of interest.

11.4 GMACE MODEL – YUONG BULLS

With the arrival of genomic evaluations in various countries, there has been a growing interest and demand for the international marketing of semen of young genomic bulls. This creates the same problem that originated before the development of MACE. The fact that proofs for progeny proven sires are not comparable across countries also extends to genomic young sires. For this reason, INTERBULL launched from August 2014 a new official service, referred to as Genomic MACE.

Genomic MACE evaluations use the national genomic evaluation estimated in each of the countries where a genotype exists and produces a genomic evaluation on the scale of the country that do not yet have a genotype. This service will help the Italian breeders to assess the genetic merit of young bulls belonging to countries not part of the consortium Intercontinental (United States, Canada, Great Britain and Italy).

11.5 How it works

Every country that wants to participate in GMACE evaluation send to INTERBULL national genomic index for all the characters, which it has passed the validation genomics. Currently Italy has validated all the characters for which it is already the official national genomic index (production, morphology and functionality). It goes without saying that the ideal scenario would be to have a direct exchange of genotypes among all countries, as is already the case within the countries belonging to the consortia Intercontinental (United States, Canada, Great Britain and Italy) and EuroGenomics (France, Scandinavia, Germany, Spain, the Netherlands/Flanders and Poland). Estimation of genomic breeding values, starting directly from the genotype of each animal is much more reliable than a pedigree index, especially for young bulls (no available info on their daughters) or when the number of daughters is reduced. Figure 6 shows the level of the indices pedigree and genomic in different groups of traits.
11.6 What is published

INTERBULL computations give back to each Country the breeding value of all the bulls evaluated in the World (around 70,000) on the Country scale. Each Country has approved rules of publications of those proofs. These rules are subject to changes and the rules up to date are always published on the WEB site in the area related to breeding values. In August 2007 in Italy rules of publications were as follows:

PRODUCTION - CONFORMATION - LONGEVITY - CALVING EASE - INTERBULL proof is official only for foreign bulls.
**GENOMICS GLOSSARY**

**Allele**: a particular DNA variant that may be responsible for alternative forms of a trait. Every individual has a paternal allele and a maternal allele, which may be the same (homozygote) or different (heterozygote).

**DGV**: direct genomic index (the simple sum of the effects of markers).

**DNA**: (deoxyribonucleic acid). The substance that makes up the gene pool. It is formed by a sequence of 4 nucleo bases.

**EBV**: traditional index.

**GEBV**: the combination of a traditional index obtained using information on daughters (EBV) and a direct genomic index (DGV).

**Gene**: the basic physical unit of heredity. A segment of DNA that codes for a function or several correlated functions.

**Genome**: the set of chromosomes contained in every cell in an organism.

**Genomics**: the field of molecular biology that deals with the study of the genome of organisms.

**Genotyping**: The process of determining a genotype by analyzing DNA. A specified sequence is compared with another. SNP genotyping measures genetic variations (alleles) in the polymorphism of a single nucleotide (SNP).

**Genotype**: The set of genes that make up the DNA of an organism or population.

**Imputation**: Methodology used to estimate missing parts of a genotype using information from individuals in the same family.

**Molecular markers**: Pieces of DNA whose alleles can be easily identified in the laboratory. They are used to identify neighboring genes.

**Nucleotide**: The building block of DNA.

**SNP molecular marker panel**: A laboratory kit used to determine the genotype of a certain individual (genotyping). These kits may contain different quantities of information. The most common is 54K, which contains approx. 54,000 pieces of information. The most recent are HD (High Density) panels, which contain around 800,000 pieces of information, and LD (Low Density) panels with around 7,000 pieces of information.

**Polymorphism**: The simultaneous presence of two or more alleles or nucleotides in the same population.

**SNP**: single nucleotide polymorphism. Polymorphism in a specific base or nucleotide in the DNA sequence; for example, at a point in the DNA sequence where one allele has one A and another allele has one T.

**Genomic proven sire**: A sire with daughters whose final index (GEBV) is the result of combining the traditional index (EBV) with the direct genomic index (DGV).

**Genomic young bull**: A bull without daughters, whose index (DGV) is obtained directly from markers and from the pedigree.