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New phenotypes for new breeding goals in dairy cattle

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Cattle production faces new challenges regarding sustainability with its three pillars – economic, societal and environmental. The following three main factors will drive dairy cattle selection in the future: (1) During a long period, intensive selection for enhanced productivity has deteriorated most functional traits, some reaching a critical point and needing to be restored. This is especially the case for the Holstein breed and for female fertility, mastitis resistance, longevity and metabolic diseases. (2) Genomic selection offers two new opportunities: as the potential genetic gain can be almost doubled, more traits can be efficiently selected; phenotype recording can be decoupled from selection and limited to several thousand animals. (3) Additional information from other traits can be used, either from existing traditional recording systems at the farm level or from the recent and rapid development of new technologies and precision farming. Milk composition (i.e. mainly fatty acids) should be adapted to better meet human nutritional requirements. Fatty acids can be measured through a new interpretation of the usual medium infrared spectra. Milk composition can also provide additional information about reproduction and health. Modern milk recorders also provide new information, that is, on milking speed or on the shape of milking curves. Electronic devices measuring physiological or activity parameters can predict physiological status like estrus or diseases, and can record behavioral traits. Slaughterhouse data may permit effective selection on carcass traits. Efficient observatories should be set up for early detection of new emerging genetic defects. In the near future, social acceptance of cattle production could depend on its capacity to decrease its ecological footprint. The first solution consists in increasing survival and longevity to reduce replacement needs and the number of nonproductive animals. At the individual level, selection on rumen activity may lead to decreased methane production and concomitantly to improved feed efficiency. A major effort should be dedicated to this new field of research and particularly to rumen flora metagenomics. Low input in cattle production is very important and tomorrow's cow will need to adapt to a less intensive production environment, particularly lower feed quality and limited care. Finally, global climate change will increase pathogen pressure, thus more accurate predictors for disease resistance will be required.

Keywords: dairy cattle, phenotype, breeding objective, selection

Implication

New breeding goals should be defined in dairy cattle to face new challenges for sustainable production, to restore functional traits and to address societal demands. Genomic selection offers new opportunities, with the possibility of decoupling phenotype recording from selection. Moreover, new phenotypes can be exploited, originating either from existing traditional recording at the farm level or from precision farming based on automated recording or sensor technology. This paper describes the different opportunities to use new phenotypes in dairy cattle breeding, particularly for milk composition, health, adaptation and environmental footprint.

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Introduction

For several decades, dairy cattle have been selected with more or less the same approach throughout the world. Breeding values were estimated from performances recorded on candidates and their relatives. The basic information was therefore performances and their main known factors of variation (herd, dates, age, etc.) and pedigree relationships. The situation was quite simple in dairy cattle with the predominant use of artificial insemination (AI) with frozen semen. Selection was mainly organized around the progeny test, which was the only method providing the reliability required for the widespread use of AI bulls, because the most important traits were recorded only in terms of female traits (milk production, udder conformation, female fertility, mastitis resistance, etc.). Therefore, traits of interest included

in the breeding objective had to be recorded on a large scale in many commercial herds. These constraints, that is, large numbers, low cost, multiplicity of organizations involved and ease of practice limited the number of traits recorded and selected in the recent past. Nevertheless, the number of recorded traits has gradually increased over time, and now up to 40 traits relative to milk production and composition, type, reproduction and health are commonly recorded in dairy cattle (Banos, 2010).

Selection is becoming more complex to meet new challenges. World's human population and urbanization are expected to increase and this will increase the demand for animal products and also the pressure on resources (land, water, energy, etc.). This will reinforce the importance of efficiency with regard to the use of resources. At the same time, social demand is related to sustainability with animal welfare, reduction of cattle environmental footprint, land use or genetic diversity. Consumers' demand is oriented mainly toward food safety and nutritional quality of animal products. Breeders' demand is related to profitability and reduced labor per animal. Finally, some global change-related constraints will have to be accounted for in the future, with increased pathogen pressure and more extreme temperatures.

However, the emergence of genomic selection (Hayes *et al.*, 2009) provides new opportunities for major changes in dairy cattle breeding. Genomic selection has two important properties: (1) it strongly increases the potential genetic gain, by up to 80% because of a reduced generation interval (Schaeffer, 2006); and (2) it disconnects the phenotype recording in a reference population from the selection of the candidates evaluated from their genomic information. This new situation is a fantastic opportunity to revisit breeding goals, because ideas that were considered unrealistic in the past may become feasible in the near future. On one hand, this increase in possible genetic gain makes room for new traits and on the other hand a trait can be selected as soon as several thousand or tens of thousand animals are recorded for this trait. This remains a large number of animals but is clearly smaller than before, and these animals no longer need to be candidates or progeny of candidates.

At the same time, the 'so-called' precision farming is developing. Implementation of electronic animal identification, development of sensor-based data and development of information and communication technologies provide opportunities for new on-farm data collection and transfer. Precision farming has the potential to increase dramatically the amount of data available.

'Omics' technologies also provide new opportunities for phenotyping. The horizon is technically quite unlimited and developments will depend only on the profitability of this information for the farmer or the breeder. Tests could characterize either the animal itself, or its microbial environment involved in nutrition as the rumen flora or in infectious diseases such as mastitis. An important limitation is the availability of the biological sample, particularly for tissues other than blood, which is difficult to obtain from live animals. The real added value of these approaches relies on our ability to transform

these raw data without interest on their own at the farmer level into informative diagnosis through indicator traits. Some of the first applications are proteomic analyses with protein chips to predict meat quality (Guillemin *et al.*, 2011), as well as different kinds of diagnostic tests for infectious diseases (e.g. Koskinen *et al.*, 2009). This huge field will not be developed in this paper.

This paper describes the different opportunities to record new phenotypes in dairy cattle breeding by making better use of data already existing for farm management or using new indicators developed through precision farming devices. The best opportunities are relative to milk composition, health and adaptation, whereas specific diseases, feed efficiency and environmental footprint are likely to remain difficult to measure.

Overview of commonly recorded traits

At present, 30 to 40 traits are commonly recorded in dairy cattle and used in selection schemes implemented in many developed countries (Banos, 2010). The first category is related to milk recording. Milk production is recorded throughout lactation and milk is sampled to be analyzed in laboratories using mid-infrared (MIR) spectrometry to provide milk composition (mainly fat, protein, somatic cells). Other optional measures are proposed; for example, for lactose or urea. These measures are performed on a regular basis generally through milk recording organizations according to several possible designs combining the following options: recording by a technician or by the farmer; recording of one or all milkings on each test day; time interval from 3 to 6 weeks between test days.

In many countries, longevity and survival are assessed through the animal traceability process: each animal, alive or dead, is identified at birth and traced on its passport throughout its whole life up to its death (natural death, slaughter), as well as its movements across farms and its possible exportation. Conditions of calving, sex, twinning are recorded at calving during the identification declaration.

Fertility-related phenotypes (conception rate, intervals, number of services, gestation length, etc.) are obtained mainly through insemination and calving data. Complementary natural mating data require a declaration by the farmer. A special information system is required for embryo transfer.

Type data are obtained through a specialized recording system. Specially trained technicians visit farms and score the animals for a number of conformation traits and survey the farmer for some additional traits such as milking speed or temperament. Additional traits are recorded by milk-recording agencies, such as clinical mastitis.

In France, all the above-mentioned traits are routinely recorded on a large scale. For example, 100% dairy cows are identified, 100% AIs are recorded, ~70% dairy cows are milk recorded and ~30% are classified for type (http://www.inst-elevage.asso.fr/spip.php?page=article_espace&id_espace=935&id_article=19072).

Many new traits could be recorded with marginal extra effort
Making better use of milk-recording data is the first opportunity and a priority. Developments based on improving the use of

milk test-day records have made it possible to better exploit lactation curves and especially persistency (Druet *et al.*, 2003). Eding *et al.* (2009) showed that mastitis occurrences could be predicted, to some extent, by somatic cell count data.

However, the most appealing development is a broader use of MIR spectra. At present, they are used to predict a limited number of milk components, especially total fat and protein percentages, but could be used to predict many more traits, as soon as prediction equations are available. After the pioneering work in Gembloux University (Soyeurt *et al.*, 2006), this approach is under study in other countries, such as in France with the 'PhenoFinlait' project (Brochard *et al.*, 2009). The principle is the following. Prediction equations need to be established by comparing MIR spectra with reference measures of a given trait for several hundreds of samples presenting a large variability. These prediction equations could then be used to predict this new phenotype on all the samples analyzed in milk laboratories (e.g. ~25 million in France) at virtually no additional cost.

Several conditions, however, need to be met in order to make this approach successful. The first one is to collect MIR spectra on a routine basis. The corresponding database is very large, as each spectrum is a plot of ~1000 (x, y) points. MIR spectra are an example of high-throughput data with regard to both the number of measures and the number of variables.

A second condition is the calibration to adjust for the drift of the analyzer and for differences across analyzers. The recommended method is to regularly analyze reference milk samples to assess these differences and adjust for them (Leray *et al.*, 2011).

The first series of candidate traits predicted by MIR are milk fatty acids (Soyeurt *et al.*, 2006; Ferrand *et al.*, 2011). Accurate predictions could be obtained for 15 to 25 different individual fatty acids or groups of fatty acids. The first results on genetic parameters show a negative correlation between saturated and unsaturated fatty acids, a positive correlation between saturated fatty acids and total fat content and a medium-to-high heritability for all components (Soyeurt *et al.*, 2007a and 2008). These estimates are very similar to those obtained with the classical methods (e.g. Stoop *et al.*, 2008) and validate the approach. Fatty acid desaturation activity could also be measured by some fatty acid ratios (Soyeurt *et al.*, 2009a). This approach provides many potential new traits and it is not yet clear how all these results should be included in the breeding goal. Therefore, additional work is needed to define what should be the objective in terms of milk composition but it is likely that fatty acids will be included very soon in the breeding goal. Encouraging results, although less accurate, were also obtained in the prediction of individual proteins (e.g. lactoferrin, Soyeurt *et al.*, 2007b) or minerals (Soyeurt *et al.*, 2009b).

MIR spectral data are also accurate predictors for parameters related to milk processing properties such as coagulation, acidity and pH (Dal Zotto *et al.*, 2008; De Marchi *et al.*, 2009). Cecchinato *et al.* (2009) demonstrated that selection for milk with better cheese processing ability predicted from MIR data is feasible. Finally, it should be emphasized that any trait

correlated with milk composition could be indirectly predicted by MIR spectra and this opens a completely new way to implement performance recording, not only for genetic purposes but also for traits related to health or reproduction. For instance, body mobilization is correlated with unsaturated fatty acids and body condition score could be predicted by MIR (McParland *et al.*, 2011). The prediction of the reproductive status (pregnant or not) is still a very partially solved question and current methods (progesterone test, echography, rectal palpation) still lack accuracy. Its prediction by MIR in milk is under study in different laboratories. If successful, this method would provide a very useful tool to characterize fertility.

Many other traits could be recorded at a low cost. Foot infections are among the main reasons for involuntary culling in dairy cattle (Fourichon, 2001; Eriksson, 2006). Hoof trimming is a regular service provided by specialized technicians. This care is an excellent opportunity to score the health of the feet of the animals, as already implemented in some countries (Cramer *et al.*, 2008; Stoop *et al.*, 2010). A standardized scoring table has been established in France and a pilot study has been launched to collect these data on a large scale.

More generally, health traits are the major challenge in the coming years. Health is the major component of sustainable production and selection. With the notable exception of the Nordic countries, which have been recording health traits for many years, large-scale recording is limited to somatic cell counts in most countries. However, many efforts have been done recently in different countries; for example, Austria with the *Gesundheitsmonitoring Rind Projekt* (Egger-Danner *et al.*, 2010) and Canada with the National Health Project (Van Doormaal, 2009). Indeed, this health information already exists at the farm level with the sanitary notebook, an on-farm recording mandatory in many countries. Collecting this disease occurrence information at a large scale and in a centralized and normalized way would make it possible to use it in selection. Although all information is of interest, a major focus could be placed on metabolic diseases such as ketosis, milk fever, acidosis, on frequent infectious diseases such as mastitis (when not already done), lameness or metritis and on reproductive disorders (retained placenta, cystic ovarian disease).

Specialized recording systems are needed for other health traits

In future, we will need more robust dairy cows. We will also have to reduce the use of chemicals to limit environment contamination and to meet the demand of the citizens. Finally, global warming may increase the pathogen pressure, particularly for parasites and insect-borne diseases. Selection could contribute to an integrated management of health for many diseases with a complex determinism. Digestive and respiratory diseases are good targets for young animals as they are responsible for very important economic losses. However, they need a specialized recording system, in the farms and, possibly, in the slaughterhouses (Assie *et al.*, 2009). Another interesting target is paratuberculosis. This disease is very difficult to eradicate as the pathogen can remain for a

very long time on the farm and the diagnosis, based on Elisa and PCR tests, is still quite difficult (Collins and Manning, 2005). An accurate phenotyping requires different tools and a longitudinal analysis over several years. In spite of the difficulty to generate large samples, the first estimates of genetic parameters (Gonda *et al.*, 2006) have been published, as well as the first Quantitative Trait Loci detection results (Minozzi *et al.*, 2010). According to these results and the knowledge in man and other species for similar diseases, it is likely that paratuberculosis has a large genetic determinism. If confirmed, genomic selection could be a useful tool to fight against this very costly pathology at the expense of an important phenotyping effort.

Genetic defects

All dairy cattle breeds are genetically small populations. Because of the initial bottleneck at breed creation in the last century and to the very limited number of artificial inseminated bulls widely spread, French breeds have an effective size ranging from 20 to 50 (Boichard *et al.*, 1996) and the situation is not different in the other countries. Even the Holstein breed has a limited genetic size at the worldwide level because of the extensive spreading of the North American germplasm in the 1980s (Miglior, 2000). This low size generates an inbreeding increase rate of $\sim 1\%$ per generation and is the cause of recurrent emergences of recessive defects. Each breed has its own defects and their frequency, initially at a low level, may rapidly increase when a popular ancestor is responsible for a large proportion of the population genes and is found to be a carrier.

Recent molecular tools (single nucleotide polymorphism genotyping and sequencing) allow rapid mapping and identification of the mutation (Charlier *et al.*, 2008). Homozygosity mapping is a method based on the search of a chromosomal region always homozygous in the affected animals, whereas rarely homozygous in the population. When used on several individuals, it can map the defect in a region of one or several megabases. New sequencing technologies, when applied to captured regions or even to the whole genome make it possible to identify the causative mutation. With a test for the causative polymorphism, the defect could be eradicated at a limited cost, by selecting only noncarrier young males as future AI bulls, even when its frequency in the population is already quite high.

This strategy requires that affected calves are detected early, accurately characterized at the clinical level by veterinarians and that tissues are sampled for DNA analysis. An observatory should be set up in order to systematically detect early emerging abnormalities, at birth or later in life. Such an observatory should involve all the organizations in close contact with the farmers, particularly veterinarians and AI technicians, as well as geneticists for genomic analyses. The Danish observatory (Agerholm, 2007) proved to be very efficient to detect emerging defects and to characterize them. The French National Observatory for Congenital Abnormalities was set up in 2001 after the emergence of Complex Vertebral Malformation syndrome in Holstein and is still very active. It has been shown

to be successful to detect different syndromes such as caprine-like generalized hypoplasia syndrome, atresia coli or different ataxia (Capel and Duchesne, 2010).

The situation is quite similar for the trait 'presence/absence of horns'. In modern dairy production systems, horns are not desirable and dehorning is practiced almost systematically at a young age. However, this practice is criticized by many welfare organizations and may be forbidden in the future. A genetic alternative is well known with the polled gene mapped on chromosome 1 but not yet fully characterized. It is a dominant gene with no known adverse pleiotropic effect in contrast with other genes with comparable effects (Capitan *et al.*, 2011). The polled gene, however, is not observed in all breeds. When genetic introgression with successive crossbreeding generations is not accepted, the only alternative is to carefully search for naturally occurring polled calves without any associated disorder.

Carcass and meat quality traits

In most European countries, a complete traceability of animals and meat has been set up after the mad cow disease crisis. Increasingly, cuts are matured and meat pieces are prepared in the slaughterhouses and delivered ready for sale in the supermarkets. In theory, the combination of these two characteristics makes it possible to accurately know not only the carcass weight and its classification, but also the meat yield of each animal, the percentage of the different cuts and possibly the maturation time required per animal, a very important parameter for meat tenderness. This strategic information already exists in some slaughterhouses; however, to our knowledge, its use in selection has not yet been studied. Alternatively, digital images can be used to predict carcass cut yields (Pabiou *et al.*, 2011). Tenderness could also be predicted by biochemical markers and protein chips are presently under development to quantify several hundreds of proteins simultaneously (Guillemin *et al.*, 2011).

Three dimensional (3D) imaging

New imaging technologies are expected to provide interesting solutions to old and only partially solved questions. Tomography is developed for smaller animals but is not adapted to the large body size of cattle. 3D imaging could provide a very accurate description of the exterior body surface at a reasonable cost. It could be used to predict many morphological indicators. As already mentioned, it could be used to predict carcass cut yields. In live animals, a trait of special interest is body condition score (Bewley *et al.*, 2008; Halachmi *et al.*, 2008) because of its association with productivity, health, longevity and welfare (Roche *et al.*, 2009). Imaging can accurately detect and measure angularity, and intensive work in this field is currently being carried out.

Precision farming

Electronic devices are more and more present on the farm. The 2010 North American Conference on Precision Farming of Toronto (<http://www.precisiondairy2010.com/pdm2010conference>) provided a complete picture of this

rapidly evolving field. In most cases, they include a recording instrument (physical, chemical, optical, etc.), a system for data recovery and an interpretative and diagnostic software. They could be set up nearly anywhere, at key locations on the farms, on the cow and even in the cow. Of course, all devices require electronic identification of the animals.

The earliest devices have been set up in the milking parlor or in the milking robot. They analyzed different milk characteristics and produced indicators based on temperature, conductivity, milk quantity, milk composition, production balance across quarters or milking flow. Typical phenotypes produced were relative to milk yield and composition, mastitis, milking speed, as well as indicators of their evolution over successive days. Some devices such as Herd Navigator take samples and include an on-farm analyzer for a more accurate diagnosis with biological markers such as lactate dehydrogenase for mastitis (Friggens *et al.*, 2007; Hojsgaard and Friggens, 2010), beta-hydroxybutyrate for ketosis and progesterone for reproduction.

In many farms, concentrate is distributed individually by feeding machines, and concentrate consumption and feeding times are recorded. Roughage consumption is still very demanding to record, in terms of investments and labor, and is reserved to experimental facilities.

Automatic weighing several times a day is a promising way to predict the daily weight change, the random variations due to feeding and drinking being controlled by the accumulation of measures.

Cows can be equipped with other devices. Efficient heat-detection systems are already in the market and other systems can inform the farmer about calving, cardiac rhythm, chewing activity or cow activity. Some devices are encapsulated in bolus and put into the rumen. When the cow is close to a receptor, the bolus can transmit information about past temperature.

Potential applications are virtually unlimited to characterize health, behavior, reproduction, production or feeding. Their development depends either on their economical interest for the farmer or the interest of the breeding company to contract with the farmer to collect information. An important challenge is the access to these data, as most of these devices have their own software and do not always allow data exports. Another major point is data quality and standardization, and the International Committee for Animal Recording is an appropriate and independent organization to define the guidelines.

Environmental footprint

Since 2006 and the 'Livestock's Long Shadow' report (Food and Agriculture Organization, 2006), cattle are criticized for contributing largely to overall green house gas production, particularly through the methane produced by their rumen fermentations. Although many of these arguments have been discussed (see for instance the analyses of Pfimlin, 2008 or Mitloehner, 2009) and sometimes revoked, the image of cattle production has been deteriorated and methane production is likely to become and stay as a trait of large interest in the next decade. Consequently, although

green house gas has presently no market value and, therefore, no economic weight in the breeding objective, decreasing its environmental footprint is a major challenge for the cattle industry. Of course, there are many ways to decrease the environmental impact of dairy cattle and a complete diagnosis at the production system is necessary to define the best action plan (Martin *et al.*, 2010; Bertrand and Barnett, 2011). In developed countries, the most important one is probably to increase longevity in order to decrease the replacement rate and the number of nonproductive animals. When production level is low, increasing productivity could reduce environmental footprint per production unit. However, a direct action on methane production should not be excluded. Indeed, there is an important individual variability in methane production, even for a given feeding system. At a given production level, this variability is related to feed efficiency: methane production corresponds to a loss of energy and is negatively correlated to feed conversion. Limited data in the literature, mostly in beef cattle, show that methane production is genetically determined and is related to feed efficiency (Hegarty *et al.*, 2007). The first step is to define the most relevant traits and the way to measure them. Methane production could be directly recorded by analyzing the gas produced by the cow. At least two approaches have been used: in the first one, the gas produced is collected in a bottle and methane is measured by comparison with a reference gas (SF₆) delivered at a constant rate by a bolus put in the rumen (Pinares-Patiño *et al.*, 2003); in the second approach, the cow is placed in a respiratory chamber. The principle of the respiratory chamber was recently adapted to the milking robot. Although the robot is not fully confined and the cow is present only for a short time, Madsen *et al.* (2010) showed that methane production could be measured by MIR by comparing methane and CO₂ concentrations. This approach could be routinely implemented and provide repeated measures for each cow at each milking, as shown by Lassen *et al.* (2010). Measuring feed consumption and residual feed intake provides more information but is much more demanding in equipment and labor than methane recording. Even if these equipments are improved and simplified, these measurements will be reserved to experimental facilities. In the future, metagenomics is expected to provide a direct measure of rumen flora composition and their control by the cow and to predict fermentation processes. In any case, this type of phenotype will remain very demanding. If a large reference population is required for genomic selection, the most appealing way would be to produce such a population at the international level through a large collaboration.

Toward a new organization of selection and data recording?

Genomic selection is a revolution and the major changes will affect data recording. Selection will need more diverse phenotypes, which is individually more expensive and is recorded on fewer animals than now, and fewer farms will be involved in the production of these new phenotypes. Breeding organizations will need reference populations of 20 000 to 30 000 recorded animals and will contribute

to data collection more than now. Because selection and phenotype production will be more tightly linked, future breeding organizations may incorporate these two activities. International collaboration will be necessary for traits difficult and expensive to measure and for sharing reference populations but it may be hampered by the strategic importance of the phenotypes. For a breeding company, having access to some rare phenotypes is a competitive advantage. However, as soon as these new phenotypes are frequently recorded, there is a clear advantage to share them in order to increase the overall accuracy of genomic estimated breeding values and decrease the phenotyping cost. Breeding objectives are evolving everywhere throughout the world with an increasing weight of functional traits. This trend will continue and additional traits may be included in the breeding goals. The role of the dairy factories is not clear but they will influence selection at least through the price of the products and, therefore, through the definition of the breeding objective and especially for fine milk composition. The large potential genetic gain will favor customized objectives according to farms or environments. A critical question concerns genotype \times environment ($G \times E$) interactions. With a limited reference population in a given environment, selection would be more sensitive to $G \times E$ than traditional progeny tests with daughters distributed in a large number of herds. Therefore, it is recommended to produce phenotypes over a range of environments.

Conclusion

In the last 40 years, phenotype recording was oriented by the constraints of selection. Phenotypes had to be recorded on a large scale and at low cost in order to be compatible with progeny testing. Past selection has put too much emphasis on production traits, in a nonsustainable way, and at present need to change its goals. Cattle selection will incorporate new traits more easily than in the past. Indeed, genomic selection relies upon a reference population of animals different from candidates but the expected genetic gain is strongly increased, offering room for new traits.

Many new phenotypes could be easily recorded because they potentially already exist and just require normalization, coordination between partners and centralization in large databases. Precision farming also provides many new opportunities, as soon as the corresponding new phenotypes can be accessed for selection. Many efforts are made to characterize fine milk composition in different countries and these traits are likely to be the next candidates for selection in the coming years. Important progress is also expected in recording health traits, at least for disease events.

Although the main challenge at present and in the near future is phenotyping, data collection has to be revisited in a completely new way, likely with fewer farmers providing data, probably under a contractual basis, and new recorded traits relative to animal health, societal demand or adaptation to global change.

High-throughput has become a very popular word. It could be interpreted in three directions: number of animals, number of traits and at different scales from the molecule to the animal. Selection will still require many animals, at least several thousand even with genomic selection (Hayes *et al.*, 2009) and these numbers will be the most serious constraint in the future. Therefore, considerable effort should be dedicated at the international level not only to develop these high-throughput methodologies but also to make them applicable to a large population. One of the first constraints is to facilitate the merging of databases. A standardized definition of phenotypes of interest is a prerequisite, as proposed by the animal trait ontology for livestock projects (Hughes *et al.*, 2008; Hurtaud *et al.*, 2011).

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