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How Could Plant Breeding Contribute to Improve Poultry Nutrition?

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Variety breeding is a slow process and it is important to identify the breeding stakes. In cereals and protein crops, high and reliable yields, high dry matter and protein digestibility and low environmental impacts are the main stakes for breeding. The analysis of the breeding achievements shows the constant genetic gains, but is not relevant with the actual yield in farming conditions, probably because of more hectic weather conditions during seed filling periods. This points out the resistance to drought stress and more generally the adaptation as major objectives. Conventional breeding, including molecular resources is presented for cereals and protein crops showing the major breakthrough over the last decades and the large potential offered by a better understanding of genome structure and gene function. Gene transfer technology is a complementary option. The methodology, possibilities and limitations of this technology are presented and the traits which could be modified and improved are briefly reviewed. Breeding improved varieties requires long term goals, and because of its very low cost, it has a major potential for the end-users.

Keywords: genetic gains, adaptability, drought resistance, molecular biology, GMO

Introduction:

At the European and international scale, agriculture and all its components must show a high economic and environmental performance. Production and farm economic performance have long been the only stakes of agriculture. It is obvious that the tremendous uptake of agriculture and associated industries has made it possible to supply a high level of food to most of the world population thus meeting the increasing human population and poultry meat consumption, with a satisfactory sanitary level. It is also obvious that the implemented farming systems had severe environmental consequences, especially nutrient losses, greenhouse gas emission and loss of biodiversity (Steinfeld et al, 2006). Poultry production meets similar concerns and all its components have to be questioned on their contribution to both economic and environmental performance. Poultry nutrition has made huge progresses over the last decades. Thanks to optimised feed composition, it made it possible to reduce the feed cost, to increase the animal weight gain and to reduce the environmental impacts. However, this supply chain is sometimes questioned and social acceptance of poultry production must be improved. Plant breeding is one step upstream as it may contribute to the quality of the raw material entering the feed processing industry. The challenges for plant breeding and the breeding objectives have to be discussed at the light of the stakes of the whole supply chain, from feed processing industry, to farmers and down to the end-users, i.e. the consumers. The crops to be considered are the cereals and protein crops.

This led us to identify three major stakes for plant breeding and one challenge. The stakes are 1) a low cost of feed. This means that high production of grains per unit area, high metabolic energy per kilogram and low production costs. This has been the main objectives over the last decades and we will document the achievements in this domain; 2) low environmental impact. The main consequence in terms of plant breeding is the high digestibility of the raw material, and as a consequence a low amount of manure; 3) reliability of volumes. This is clearly a challenging objective and a barrier to innovation. Indeed, due to the performance of feed industry, large volumes with a stable chemical composition are compulsory conditions to access the market. As a consequence, it is difficult to market new species or varieties with very different features.

Plant breeding also faces another challenge which depends very much on the country. Indeed, the social acceptance of some breeding technologies may be poor. It is for instance the case of transgenesis technology. This will be discussed in the last part of the paper.

Breeding achievements in the past decades

Plant breeding is a slow process. Its speed depends on the species and the breeding methods. But, for annual grain crops such as winter cereals or field pea, more than ten years are needed between the initial crosses and the variety registration. The consequences are that i) the presently observed genetic gains are the results of the objectives assigned some ten years ago, ii) the conditions, either due to climatic conditions or to agronomic practices may be different from the anticipated ones and iii) consistency of goals is required.

Focussing on winter cereals and field pea, we will analyse genetic gain on various traits and the actual consequences in agriculture.

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Case study of wheat on the last three decades

Several studies have been carried out to measure the genetic gain in winter cereals and published over the last few years. (Donmez et al, 2001; Brancourt-Hulmel et al, 2003; Austin et al, 1989; Underdahl et al, 2008)

We will illustrate the progress through the results of variety registration trials run in France over the last decades, the French catalogue being one of the main European national catalogue for wheat.

As it can be seen on Figure 1 on data from standardized registration trials, the mean grain yield of the varieties has steadily increased over the last four decades. The genetic gain is slightly higher in absence of pest and disease protection than under pest and disease protection. This increase is due to the facts that yield was the main registration criterion and the main breeding objectives of the numerous wheat breeders. This was achieved through the modification of different yield components, architectural modifications (especially shorter plants which are more resistant to lodging) and physiological improvements (higher nitrogen efficiency, higher harvest index¹, disease resistance). In the same time, a decline in protein content was observed (figure 2). The reason of this decline may be a dilution effect related to yield improvement, as the nitrogen pool available in the sward is 'diluted' in a larger grain biomass. In real agronomic conditions, the protein content may even be lower if the nitrogen fertilisation is below the optimum level (critical nitrogen curve), this situation affecting both the grain yield and the protein content (Lemaire et al, 2008).

Figure 1: Mean yield of registered varieties in registration trials under low disease protection from 1970 to 2007. The mean genetic gain is 0.13 t/ha/year. (Source: G. Charmet)

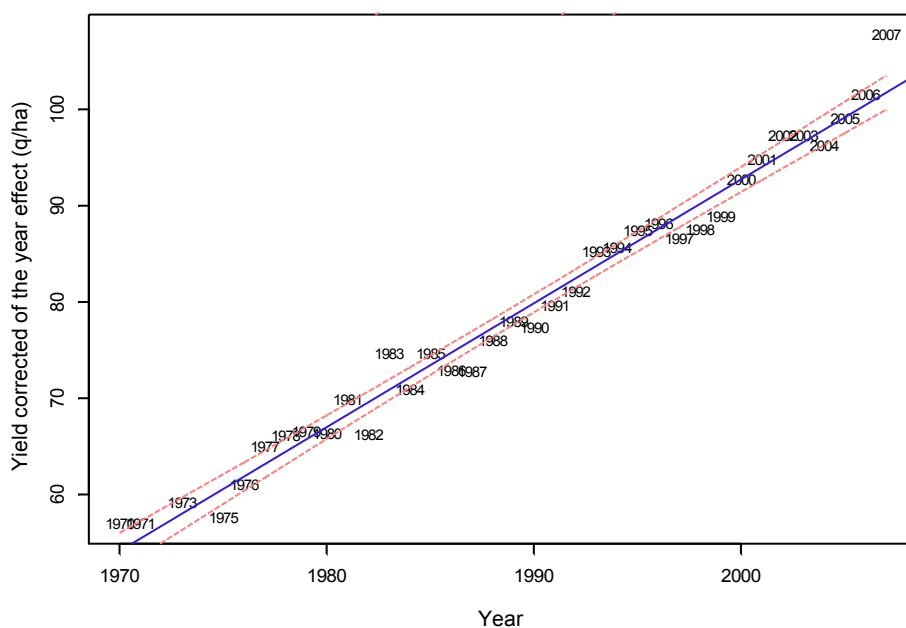
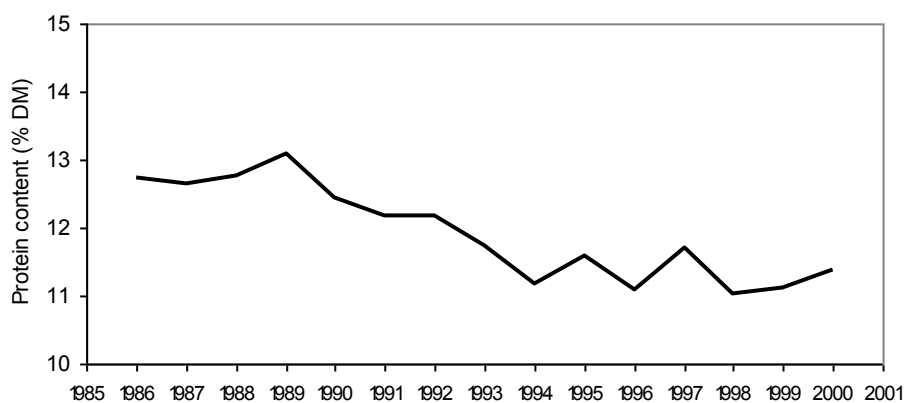


Figure 2: Mean protein content of registered varieties in registration trials from 1986 to 2000. The x-axis indicates the year of application (Source: A. Luciani, GEVES)



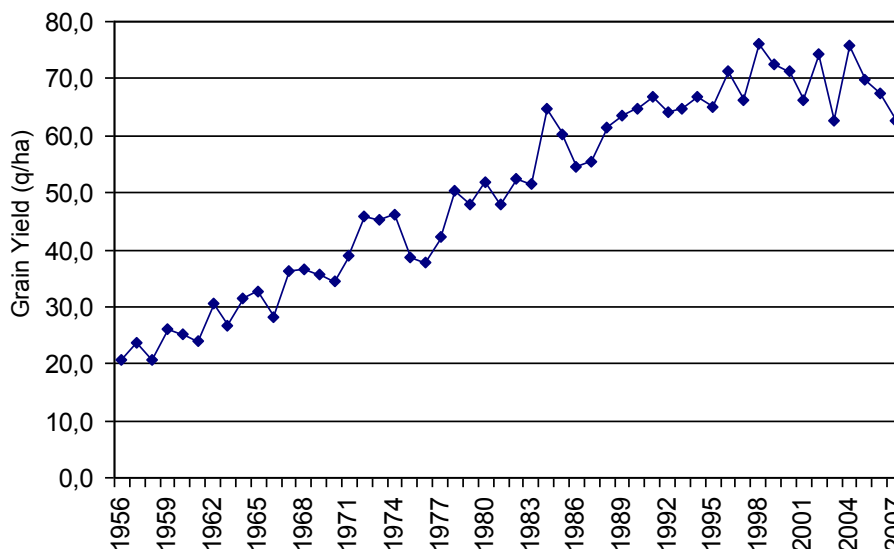
¹ Harvest Index (HI): ratio between grain yield and total plant biomass at harvest.

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The main consequence of this decreasing protein content will be the need for a higher supplementation with protein crops, such as soybean meals or with industrial amino acids. This may be impossible in some production systems, such as organic farming.

It is interesting to show in parallel the actual grain yield of wheat (Figure 3). The mean yield increase between 1976 and 1990 was 0.18 t/ha/year. It is intriguing to see that over the last ten years the wheat actual yield did not increase, while the genetic potential of the varieties increased. Similar features are seen in most European countries. The reasons for this stability are still debated. Among the possible reasons, it is proposed that the mean nitrogen fertilisation was sub-optimal due to low grain prices and high disease pressure. It is also suggested that this may be one of the first consequences of the global warming which tends to shorten the favourable growing season of winter wheat in France. The main changes presently observed are a reduction of rainfall at the end of spring and an increased evapotranspiration during this period which is critical for grain filling. This feature would be relevant with the continuous progress in maize which is little affected by the higher mean temperatures. No global data are available for the grain chemical composition of collected grains. However, this could have important impact on the quality of the raw material entering feed industry? This pattern of lack of genetic gain and variable yields and possibly chemical composition due to changing weather conditions is a major challenge for the breeders.

Figure 3: Actual mean grain yield of wheat in France over the last four decades. It clearly shows the absence of genetic gain during the last ten years.



Case study of protein crops in Europe

Field pea acreage has declined a lot in Europe over the last ten years because of low market prices. Despite of this situation, large genetic gains have been made. This is especially the case for mean grain yield and the crop harvestability. Indeed, peas tended to severely lodge during ripening. This was negatively affecting the harvest, with a negative impact on actual yield, grain quality and farmers work load. Breeding made it possible to select varieties which remain high at the harvest time. This contributed to higher yield and better grain quality..

The main concern today in pea breeding is the resistance to a soil born disease, *Aphanomyces euteiches*. This disease strongly affects seed yield. Today, no variety has a sufficient level of tolerance to be cultivated in infected soils. As a consequence, the pea acreage in France and Europe strongly declines. This may lead in the future to a unreliable supply. Breeding for resistance is difficult in absence of true resistance. The present research program focuses on a model legume species, *Medicago truncatula*, in order to understand physiological mechanisms, to identify resistance gene and investigate other breeding methods.

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As most grain legumes, grains of pea (*Pisum sativum*) may carry antinutritional factors (ANF), which belong to the group of antitrypsins. Breeding has removed these factors from modern pea varieties and this trait is taken into consideration for registration. In other grain legume species such as soybean (*Glycine max*), antitrypsin factors are removed through the industrial process of oil extraction, where the high temperatures destroy the ANF. Breeding for low ANF content was very successful in fababean with creation of varieties free of vicin and convicin.

What is possible in classical breeding?

In this section, we will review the possibilities to breed new varieties through classical breeding to meet requirements of poultry nutrition. We will cover the traits of interest, the existence of genetic variation available for breeding and the possibilities offered by the recent advances in molecular biology. Again, in this section, we will mainly focus on wheat and pea.

Important traits for agronomic and use value of varieties

As mentioned earlier when analysing the yield variation over the last decades, yield remains of key factor for income of producers and for the access to feed processing industry. But, beyond mean yield, the yield stability must be considered. This means that the variety x environment interaction must be taken into account when breeding or registering varieties. In wheat, it has been documented that large differences exist among varieties for their contribution to G x E interactions for grain yield in bread wheat (Brancourt-Hulmel et Lecomte, 2003) or in durum wheat (Annichiarico et al, 2009). This leads to the concept of broad adaptability of varieties, where phenology (flowering date), morphology (height, lodging resistance) and physiological features (resistance to drought) appear to be key traits. For cereals, these points were recently reviewed by Araus et al (2008). In pea, the growth cycle of spring-sown pea is short and thus very susceptible to poor weather conditions. So, in order to improve the adaptability, winter-sown material were developed. In the recent years, genotypes with a different response to daylength and new alleles for flowering dates were developed with an adequate frost resistance (Lejeune-Hénaut et al, 2008). Even though these new ideotypes still show a low mean yield, they open new prospects for breeding varieties with higher and more stable grain yields.

The modification of chemical composition of proteins has often been considered as a possible breeding objective. However, the low cost of synthetic amino acids and the lack of genetic variation must be considered. The pattern of grain storage proteins has been investigated in pea. It appeared to be difficult to exploit. Indeed, the abundance of the various types of proteins offers little prospects for modification of the content in the amino acid and especially lysine and sulphur amino acids.

Dry matter and protein digestibility by poultry is a very important trait and was investigated. Differences among varieties were found for amino acid digestibility, with average values ranging from 79.5 to 86.3% depending on the genotype (Gabriel et al, 2008 a) and was negatively correlated to insoluble fibre fraction and trypsin inhibitor activity (Gabriel et al, 2008b). Insoluble fibre fraction is of peculiar importance in pea. Indeed, as cotyledons contribute a major part of the grain, cell walls with a high content in cellulose are present and decrease the possible feeding value.

Low content in phytic acid may improve digestibility of micronutrients such as Zn for monogastrics and reduce P losses in faeces (Hill et al, 2009). Genetic variability for phytic acid content was investigated in several species and genotypes with low phytic acid content were identified in soybean (Israel et al, 2005), wheat (Guttieri et al, 2006), and maize where several genes involved in the phytic acid biosynthesis pathway were identified (Lin et al, 2005; Shi et al, 2005)

Variability must be available within species or in related species

Breeding is only possible if genetic variation is available and may be exploited in breeding. Theoretically, this variation may be in the genetic resources of cultivated species or in related species. In practice, for species with a long breeding history, it proves difficult to exploit the diversity available in related species. Indeed, as it is the case for wheat, the related species which may be used for inter-species hybridizations have a low agronomic value *per se* and the long distance crosses often generate sterility or poor fertility. It is then necessary to transfer the piece of genome of interest through a very long process of backcrosses. This was for instance successfully done for disease resistance in wheat with a resistance gene from *Aegilops ventricosa*. However, more than 30 years were needed to properly integrate the small targeted fragment of chromosome. In the case of pea, no related species may be used for inter-species cross.

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Molecular genetics and plant breeding

Advances in plant molecular genetics and genomics offer new possibilities and prospects. Genomics resources are very numerous and valuable. Such resources are for instance mapping populations of RILs², insertion mutants³, linkage maps saturated with markers, QTL⁴, genome sequences. This opens possibilities to run marker-assisted-selection which may speed up the breeding process and help in focusing field trials on the most promising material. It also makes it possible to identify candidate genes which may explain the detected QTL. Once these candidate genes have been validated, it is possible to screen and use the allelic variation available within the species. This molecular research may be performed either on the agronomic species or on model species. Such model species are rice for the cereals and *Medicago truncatula* for grain legumes.

Numerous linkage maps were developed and QTL identified in wheat, barley, maize, soybean and pea. In wheat, QTLs were detected for most agronomic traits, even though each QTL only explains a small proportion of variation for yield and yield components. QTLs were also detected for disease resistance, such as powdery mildew (Muranty et al, 2009) and for chemical composition of grain (Prasad et al, 2003). In pea, QTLs were identified for disease and frost resistance, for flowering earliness, frost resistance and for quality traits (Lejeune-Hénaut et al, 2008; Timmerman-Vaughan et al, 2005).

GM technology: dream or nightmare?

The GM technology opened a huge range of theoretical possibilities, as it makes it possible to exploit the genetic variation available in all living organisms. It is thus possible to express in plants genes from other plant species, from animals, bacteria, yeasts or viruses. Two main groups of technology may be used in plants. It is either a transfer with specialized soil bacteria, belonging to *Agrobacterium* genus. These bacteria have the natural ability to insert genes from their plasmid (a circular DNA strand) into the plant genome. The insertion is usually made in the correct reading frame and only a low number of copies is inserted. The use of this technique is restricted to dicots, as monocots are resistant to these bacteria. The other technology is based upon the particle gun. The DNA to be transferred is coated onto gold or tungsten particles. These particles are then shot into tissue cultures. When particles get across cells, DNA may be deposited and DNA sequence integrated into plant genome. In this case, insertion occurs at random and high number of copies may be inserted. After insertion, transformed cells must be selected (thanks to the presence of selection genes such as resistance to antibiotics or herbicide into the transferred piece of DNA), whole plants have to be regenerated and gene expression checked. Only a low proportion of regenerated plants has the expected level of expression and this leads to a low number of successful transformation events.

These technologies show limitations, the main one being the small size of DNA fragment which may be transferred thanks to this technology. Indeed, beyond a few Kb (3-6 Kb), the DNA tends to be unstable and to break. As a consequence, in most cases, transgene only includes a selection gene, a promoter and the gene of interest with sometimes a tissue addressor. This means that traits controlled by single genes will be the main targets of GM technology. This may be the case when the trait of interest does not exist in the species (i.e. resistance to an herbicide such as glyphosate or to pest such as Bt gene against cornstalk borer (*Ostrinia nubilalis*), when an active gene has to be knocked out (antisense strategy, such as for lignification pathway) or when a regulation gene is involved (Myb and Myc genes). For complex quantitative traits, GM technology is difficult to be used and this is demonstrated by the fact that most GMOs available worldwide carry single genes controlling resistance to herbicide and to insects.

This technology also raises questions regarding the environmental risks and especially the transmission of the transgene to wild populations of cultivated or related species. The risk increases with the out-crossing rate of the species (low for cereals, peas and soybean, high for rapeseed and maize) and the presence of these wild populations (high for rapeseed). The social acceptability of this technology is presently low in Europe, but this may change if the transformed trait is of interest for the end-users. Eventually, the co-existence and traceability of transgenic and conventional productions at field scale as well as in the processing and distribution steps is strongly questioned (see Co-Extra UE project, www.coextra.eu).

Based upon this analysis, it is possible to review the traits which could be modified in the various species which are to be used in poultry nutrition. We will not consider here herbicide resistance.

Improving the yield potential *per se* is fairly unlikely through gene transfer due to the very high number of genes to be considered. But it must be questioned whether this technology could be of interest for drought tolerance. This was investigated in maize with some success with various genes such as Asr-1 (Jeanneau et al, 2002) or glutamate dehydrogenase (gdhA) gene (Lightfoot et al, 2007).

Modification of amino acid balance has been tested using SFA8, a gene encoding a sulphur-rich protein from sunflower. This was first successfully done by Tabe et al (1995) on alfalfa, but failed in many other species and especially grain crops such as narrow-leafed lupins.

² RILs : Recombinant Inbred Lines

³ Insertion mutants are genotypes where a small sequence of DNA has been inserted at random generating a random knock-out of genes, It is then possible to relate phenotypic traits and deleted genes.

⁴ QTL : Quantitative Trait Loci

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Endogenous antinutritional factors are easily modified through classical breeding thanks to the existence of natural mutants. This was for instance the case for lupins where mutants free of alkaloids were extensively exploited in breeding. But, in the case of mycotoxins, the situation may be very different. Indeed, these toxins may be present in substantial amounts in harvested, especially after disease or pest attacks or poor storage conditions and may cause very severe damages in animal nutrition. It is thus of interest to reduce it. In maize, it was shown that after plant attack by cornstalk borer, the content in mycotoxins due to secondary attacks by *Fusarium* (especially fumonisin, zearaloxone and deoxynivalenol which are regulated) may become high and the harvest has to be destroyed. It was shown that Bt transformed maize had lower cobs damaged by *Fusarium moniliforme* and mycotoxins than untreated controls (table 2).

Table 2: *Fusarium* damages and mycotoxin content in untreated conventional maize and Bt maize in situations of low and severe cornstalk borer infestations.

	Low infestations		Severe infestations	
	Cobs with <i>Fusarium moniliforme</i> (%)	Fumonisin (ppb)	Cobs with <i>Fusarium moniliforme</i>	Fumonisin (ppb)
Untreated controls	17.2	1200	20.8	3150
Bt maize	12.2	700	6.7	1300

Conclusions

Breeding is a long and slow process and genetic value of varieties is only one of the components that determine the quantitative and qualitative value of the harvested grains. The choice of varieties is one resource of feed industry alongside with processing and choice of species or other raw materials. But, the choice of improved varieties has no cost, and thus, it can easily contribute to the sustainability of this industrial sector. New molecular resources and biotechnologies are likely to speed up the breeding processes, but they will always take time. As a consequence, it is necessary to have long term goals and consistency in these goals. Securing the production volume through varieties better adapted to summer drought and stressful environmental conditions during grain filling is likely to be a major challenge for breeding during the next decades. Another challenge may be to face the reduction of species where breeding programs are carried out, leading to a reduced diversity of feed resources. On a long term, this may become a limiting factor for some production systems such as organic farming.

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