Executive summary

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Certified products of a specific quality aim to meet higher standards than the minimum statutory requirements regarding health and composition. They are intended as a response to demand from consumers concerned over food safety issues. Such quality products, designed to be compatible with the environment (linking of products to the soil) and animal welfare, require specific guarantees if they are to be credible and viable. To achieve this, systems have to be set up to verify their conformity with the specific standards and also their labelling. Reliable analytical methods are an essential part of the control procedures.

Our research is focused on the "broiler", a product characterised by a variety of seals of approval and quality labels in Belgium and in Europe : quality labels (*Label de Qualité Wallon* - Walloon Quality Label, *Label Rouge* - Red Label in France, special labelling under regulation 91/1538/EEC), European regulations relating to labels of origin (*Appellations d'Origine Protégées*, protected indications of origin and *Indications Géographique Protégées*, protected geographical indications (regulations 92/2081/EEC and 93/2037/EEC)), Organic Farming (regulation 1999/1804/EEC, national legislation and private standards).

The Department QUALITY OF AGRICULTURAL PRODUCTS at the GEMBLOUX AGRICULTURAL RESEARCH CENTRE has joined forces with the UNIT OF ANIMAL HUSBANDRY at GEMBLOUX AGRICULTURAL UNIVERSITY to develop methods for authenticating meats and meat products using rapid analysis techniques (near infrared spectroscopy-NIRS) or molecular biology techniques. Verifying the conformity of poultry feed (no animal meal or antibiotics) by NIRS in conjunction with other analytical methods (HPLC) has also been studied.

During the time of the research, it was demonstrated that near infrared spectroscopy is an appropriate technique to distinguish slow-growing chickens from fast-growing ones. Discriminant models based on the analyses of chicken meat by NIRS were developed. With this fast, cheap and non-destructive technique, NIRS-models are able to authenticate a majority (> 80 %) of the samples, be it either on carcasses or on cut pieces. An animal experiment was also set up with the collaboration of the UNIT OF ANIMAL HUSBANDRY (Professor André Théwis - GEMBLOUX AGRICULTURAL UNIVERSITY) to evaluate the relation between feed composition and NIR spectra of meat. According to the results, it would be possible to detect more than 70 % of fraud cases (chickens bred in contradiction with the set of production rules including the use of slow-growing chicken strains together with a specific feeding). **Even if discriminant models developed are based on the spectra acquired on a population with a significant number of specimens (more than 150 chickens), it would be better to extend the databases and to test new algorithms (e. g. neural network analysis). A good optimisation of the technique should allow its routine application.**

One of the conclusions of the project is that PCR-RFLP is not appropriate for detecting the components of a meat mixture although it is reported as such. The fact is that though the use of universal primers does have the theoretical advantage of not having to have preconceived ideas about the possible components of the blend, this technique in fact causes a clear bias in the detection of the species in the blend. For instance, in a turkey/chicken blend, without a doubt one of the most plausible frauds, it turned out that a 10% turkey content cannot be detected by the PCR-RFLP technique. Its presence only starts to be revealed from around 25%. This is explained by the fact that the primers, although regarded as universal, amplify the chicken in preference. In order to avoid such a bias phenomenon, the use of species-specific targets is recommended. Until now, we are able to detect adulterations of chicken meat with turkey meat at levels below one percent.

Nevertheless, the development of a strategy requiring a sole PCR to detect the various animal species in meat products remains very attractive. The use of new technologies (e.g. biochips) and specific capture probes internal to conserved regions would be a solution to the bias problems described before.

With the aid of the AFLP technique (Amplified Fragment Length Polymorphism), we have succeeded in identifying two bands which in the population under study allow specimens from slow-growing strains to be distinguished from fast-growing strains. The two molecular determinants were isolated, cloned and sequenced : one is specific of slow-growing chickens (333 bp) and the second band is characteristic of fast-growing chickens (372 bp). The two identified markers are apparently correlated to growth rate. However, one may not exclude that these markers simply reflect a tight link with a particular breed used in the slow- or in the fast-growing strains. The exact meaning of these markers is unclear, the more as no homology could be found with known sequences (comparison with sequences registered in international sequence libraries) and hereby for instance give some indications of a possible link to growth. This statement does in no way diminish the usefulness of these markers (especially in so far as verifications for use of JA strain as a slow-growing strain are concerned) but could restrict their domain of application (e.g. valorisation limited to slow-growing strains resulting from a specific breed in the ascendants…).

The choice of AFLP as a technique for investigating the polymorphism of DNA was based on the fact that it does not need any prior knowledge of the genome and is reproducible. Results to date allow us to envisage with optimism the possibility of distinguishing between slow-growing and fast-growing chicken strains according to their molecular determinants. AFLP nevertheless appears to be suitable as an investigative technique for DNA, though we feel it is too cumbersome to use for routine analyses (it can take two days to get a result). **It seems an evidence that development and validation on a representative set of a fast PCR test (results within one day) based on the determinants identified by AFLP has to be considered. A test of this kind could be routinely used by a testing laboratory but requires beforehand that the exact meaning of the determinants should be delimited. This type of test would have another advantage : it could be used with processed products because it does not require, in contrast to AFLP, a good quality DNA.**

Moreover, AFLP allows virtually infinite combinations (restriction enzyme pairs, selective nucleotides). **The AFLP technique**, which our laboratory is proficient in, **can further be used for research into new discriminant bands between fast-growing and slow-growing chicken strains. Characterisation of the slow-growing strains most commonly used commercially could be envisaged in this framework. Commercial exploitation of the research results (patents, analysis kits) is not unrealistic.**

With regard to animal feed, determining the origin of particles by means of near infrared microscopy appears to be the most promising issue for rapid detection of animal proteins and fats in feed. The application of the technique is developed at the Department QUALITY OF AGRICULTURAL PRODUCTS at the GEMBLOUX AGRICULTURAL RESEARCH CENTRE. It requires to create libraries of raw material spectra which include the variety of ingredients that go into animal feed formulations.

At present, the presence of inhibitors (antibiotics or other substances) in animal feed is verified by identifying an inhibiting power with respect to various bacterial strains. Analysis of different raw materials has revealed the inhibiting action of a number of components, in particular the different ones produced by grinding cereals (bran, middlings, wheat feed).

Other matrices (lucerne meal), after aqueous extraction, reveal an area of inhibition. Bacterial strains have been isolated from samples of raw materials showing a positive result. One of these, *Bacillus licheniformis*, which was active against the test strains, is responsible for the production of a molecule used in animal feed: bacitracin. From the chromatographic tests carried out it is evident that this antibiotic is in no way responsible for the positive results. Similar results have been observed with chicken meat samples producing abnormal positive results: a strain of *Klebsiella oxytoca* was isolated from the meat, this bacterium also show a bactericidal effect on the test strains used.

An HPLC method coupled with a fluorescence detector has been developed in order to reveal coccidiostats in feed (sulphamides). The extraction developed and the HPLC parameters confirmed the purity of the peaks obtained.