Safety assessment and feeding value for pigs, poultry and ruminant animals of pest protected (Bt) plants and herbicide tolerant (glyphosate, glufosinate) plants: interpretation of experimental results observed worldwide on GM plants

Aimé Aumaitre
INRA. Saint Gilles, France

Corresponding author: Prof. Aimé Aumaitre. INRA. 35 590 Saint Gilles, France - Tel.+ 33 223 485041 - Fax: + 33 223 485080 - Email: aumaitre@saint-gilles.rennes.inra.fr

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ABSTRACT

New varieties of plants resistant to pests and/or tolerant to specific herbicides such as maize, soybean, cotton, sugar beets, canola, have been recently developed by using genetic transformation (GT). These plants contain detectable specific active recombinant DNA (rDNA) and their derived protein. Since they have not been selected for a modification of their chemical composition, they can be considered as substantially equivalent to their parents or to commercial varieties for their content in nutrients and anti-nutritional factors. However, insect protected maize is less contaminated by mycotoxins than its parental counterpart conferring a higher degree of safety to animal feeds. The new feeds, grain and derivatives, and whole plants have been intensively tested in vivo up to 216 days for their safety and their nutritional equivalence for monogastric farm animals (pig, poultry) and ruminants (dairy cows, steers, lambs). The present article is based on the interpretation and the summary of the scientific results published in original reviewed journals either as full papers (33) or as abstracts (33) available through September 2003. For the duration of the experiments adapted to the species, feed intake, weight gain, milk yield and nutritional equivalence expressed as feed conversion and/or digestibility of nutrients have never been affected by feeding animals diets containing GT plants. In addition, in all the experimental animals, the body and carcass composition, the composition of milk and animal tissues, as well as the sensory properties of meat are not modified by the use of feeds derived from GT plants. Furthermore, the health of animals, their physiological characteristics and the survival rate are also not affected.

The presence of rDNA and derived proteins can be recognized and quantified in feeds in the case of glyphosate resistant soybean and canola and in the case of insect protected maize. However, rDNA has never been recovered either in milk, or in liver, spleen and muscles tissues of animals, or in rumen bacteria. On the basis of these data, it can be suggested that in vivo tests on high producing animals are necessary and sufficient to evaluate the safety and the nutritional value of new GT plants.

Keys words: GM plants, Safety, Feeding value, Farm animals

RIASSUNTO

SICUREZZA E VALORE NUTRITIVO DI ALIMENTI MODIFICATI GENETICAMENTE PER RESISTENZA GLI INSETTI (BT) E TOLLERANZA AGLI ERBICIDI (GLIFOSATE, GLUFOSINATE) DESTINATI A SUINI, POLLI E RUMINANTI: INTERPRETAZIONE DEI RISULTATI SPERIMENTALI OTTENUTI A LIVELLO MONDIALE.

Recentemente sono state sviluppate, mediante modifica genetica, nuove varietà di piante (mais, soia, cotone, barbabietole da zucchero, canola) resistenti agli insetti e/o tolleranti a specifici erbicidi. Queste piante contengono specifici
DNA ricombinati attivi (rDNA) e le proteine da essi derivate, entrambi rilevabili. Poiché tali varietà non sono state selezionate per modificare la composizione chimica, esse possono essere considerate sostanzialmente equivalenti, per quanto riguarda i contenuti in principi nutritivi e in fattori anti-nutrizionali, alle corrispondenti varietà convenzionali. Comunque, il mais modificato per la resistenza agli insetti risulta meno contaminato da micotoxine rispetto al convenzionale. Mediante prove in vivo, sono state valutate la sicurezza e il valore nutritivo per monogastrici e ruminanti di queste alimenti. Nella presente rassegna vengono riportati e interpretati i risultati pubblicati su riviste scientifiche sotto forma di lavori in extenso (33) o riassunti (33) fino a settembre 2003. In tutte le prove, la cui durata è variata in funzione della specie animale, l’inclusione nella dieta di alimenti geneticamente modificati non ha influenzato l’ingestione, l’accrescimento, la produzione di latte, l’indice di conversione alimentare e la digestibilità degli alimenti. Inoltre, in tutti gli esperimenti, la condizione corporea, la composizione della carcassa e dei tessuti, le proprietà sensoriali della carne, nonché la composizione del latte non hanno subito modificazioni. Nessuna alterazione, è stata infine rilevata sulla salute e sulle caratteristiche fisiologiche degli animali impiegati nelle prove. La presenza di rDNA e delle proteine derivate può essere rilevata e quantificata nel caso della soia resistente al glifosate e della canola. In ogni caso, rDNA non è mai stato rilevato nel latte, nel fegato, nella milza e nel tessuto muscolare degli animali, né nei batteri ruminali. Sulla base di questi risultati viene suggerito che le prove in vivo sugli animali in produzione sono necessarie e sufficienti per valutare la sicurezza e stimare il valore nutritivo degli alimenti geneticamente modificati.

Parole chiave: Piante GM, Sicurezza, Valore nutritivo, Allevamento animale.

Introduction

Genetically modified (GM) major feed crops have been recently developed and disseminated to produce animal feeds and fibrous material. Thus, pest protected plants, maize and cotton, and herbicide tolerant soybean, maize and canola are currently grown in the American continent representing up to 70% of the U.S. planting in 2001 (Faust, 2002). Sugar/and fodder beets tolerant to different herbicides have also been successfully developed (Böhme et al., 2001). And recently, a new variety of wheat resistant to glyphosate has been developed and tested for safety in the pig species (Peterson et al., 2003).

Farm animals are currently fed soybean and soybean meal developed from genetic transformation as well as maize and maize products such as corn gluten feed and meal. Europe is strongly dependent upon the American continent for its protein requirements amounting up to 90 to 95% for soybean, 40 to 60% for maize derivatives and partly for canola grain or meal. The world production of genetically modified soybean tolerant to glyphosate is currently estimated at 68%. In addition, ruminant animals are huge potential consumers of whole pest protected and herbicide tolerant maize plants used either as fresh or ensiled forage (Aulrich et al., 2001; Barrière et al., 2001). They are also potential future users of herbicide tolerant beets (Weisjberg et al., 2001).

Besides the legislation on the authorization of the dissemination of GM crops in Europe, it is of utmost importance that animal scientists should be involved in the assessment of the safety issues associated with the use of these new plants in the diet of farm animals (Beever and Kemp, 2000) and their consequences on the safety of the food chain (Chesson, 2001). Moreover, animal nutritionists should evaluate the nutritional equivalence and the efficacy of the new feeds, in comparison with near isogenic or conventional varieties of plants (Hammond et al., 1996; Brake and Vlachos, 1998; Aumaitre et al., 2002; Cromwell et al., 2002).

Fortunately, numerous data have been obtained in national institutions and universities, realized sometimes in the frame of a dossier of authorization of dissemination of the new constructs. Since 2000, they have been mostly published in the form of original papers in the referred scientific literature or displayed at the occasion of annual meetings of scientific associations (Faust et al., 2000; Aumaitre et al., 2002). Data available for the present review included those extracted from 33 original papers or book chapters and results of 33 additional abstracts presented and discussed at different scientific meetings through September 2003. Their main purpose was to assess the safety and evaluate the feeding value of GM plants with respect to farm animals.

Chemical composition of new plants: nutrients and anti-nutritional factors
For more than a century animal scientists have considered the chemical composition of feedstuffs as a prerequisite for the evaluation of their nutritional and feeding value. Similar data have been used for the characterization of new plants and plant products to conclude on their substantial equivalence with conventional plants (Padgette et al., 1996; Sidhu et al., 2000; Aumaitre, 2002). These data are imperatively required for the expression of their potential nutritional value based on the classical determination of crude protein, fat carbohydrate, crude fiber and total ash content, leading to a first estimation of digestible and/or net nutrients for the different categories of farm animals, mainly pigs, poultry and ruminants.

**Comparative chemical composition of new plants**

New plants and their component parts including grain, leaves, stems or their derivatives have been analyzed in detail for their major constituents, total nitrogen, fat, total carbohydrates, minerals and fibrous compounds. In some cases, depending upon the source of feeds, individual amino acids, fatty acids, trace elements and vitamins have also been quantified in new feeds. As an example, the comparative chemical composition of GM maize either herbicide tolerant or pest protected (Bt) is given in Table 1. In general, there are no significant differences in the level of major nutrients expressed on the basis of the dry matter in the kernel or in the whole maize plant between the transformed plant and the near isogenic or control plants. However, variations in the chemical composition can be associated with year, climatic conditions and fertilization or soil conditions. It is consequently advisable to compare the values with data observed for similar plants and plant products and published in the scientific literature, and with the range of the feed tables.

Thus, these first basic data lead to the provisional conclusions that insect protected plants and herbicide tolerant plants are substantially equivalent to near isogenic and control plants. Additional information published, for example, on the level of expressed insecticidal proteins such as CRY1A(b) on maize resistant to the European corn borer *Ostrinia nubilalis* are also of interest. Maximum values, largely variable according to the age of the plant, amounted to only 8 nanograms per gram fresh product in the kernel compared to up to 1mg/kg of fresh product in the leaves of Bt 176 maize. This amount of expressed protein which can hover up to 9.5 µg/g fresh weight in the case of Bt MON 810 maize leaves (FSA.agbios.com. 2003)

### Table 1. Gross Chemical composition of pest resistant maize (Bt176) and glyphosate tolerant maize (GA21): values in percent of dry matter or % of total amino acids or % of fatty acids.

| Year | 1996 (a) | 1997(a) | 1998(b) |
|------|----------|----------|----------|
| Variety | Parental | GA21 | Parental | GA21 | Parental | Bt176 |
| Protein (% DM): |  |  |  |  |  |  |
| Plant | 7.58 | 7.91 | 7.45 | 7.49 | 6.0 | 6.7* (d) |
| Kernel | 10.05 | 10.05 | 10.54 | 11.05 | 9.3 | 9.5 |
| NDF(c) (% DM): |  |  |  |  |  |  |
| Plant | 40.8 | 39.5 | 38.9 | 37.9 | 38.5 | 9.5 |
| Kernel | 11.7 | 10.8 | 9.8 | 9.3 | - | - |
| Lysine (% of amino acids), Kernel | 3.09 | 3.02 | 3.02 | 3.11 | 2.9 | 2.9 |
| C18:2 (% of fatty acids), Kernel | 58.7 | 58.6 | 61.5 | 61.4 | 57.4 | 57.3 |

(a) Sidhu et al., 2000; (b) Barrière et al., 2001; (c)Neutral detergent fiber; (d) Significant difference (P<0.05) between varieties within experiment.
cannot significantly modify the gross chemical composition (Fearing et al., 1997).

Attention has also been paid to the potential effect of the genetic modification on the content in anti-nutritional factors (ANFs). Data on major ANFs of soybean resistant to glyphosate demonstrated that neither lectins, nor trypsin inhibitors (TI) content of raw beans are modified. Moreover, trypsin inhibitors are similarly destroyed during heating associated with oil extraction and preparation of soybean meal. In addition, the activity of urease measured by the variation in pH is efficiently reduced by heat treatment whatever the genotype of the soybean kernel. Also, the level of isoflavones in the kernel and in the leaf has not been affected by the genetic modification. However, the only chemical analysis considered as the basis of the substantial equivalence cannot be used to conclude to the safety of the new plant issued from the genetic modification. Substantial equivalence is not and has never been synonymous with safety, neither in Europe (Chesson, 2001), nor in the United States (Faust, 2002). Other measures on the apparent digestibility of the nutrients can help to detect unintentional effects on the bio-availability of nutrients or on the presence of potential unknown toxicants in new plants (Aulrich et al., 2001; Böhme et al., 2001).

### Table 2. Main antinutritional factors in raw soybean and soybean meal: absence of effect of genetic modification for glyphosate resistance (Adapted from Padgette et al., 1996).

| Kernel Product | Parental | Glyphosate resistant |
|----------------|---------|---------------------|
| Lectins        | Raw     | Meal               |
| HU/mg sample   | 1.2     | 1.0                 |
| Trypsin inhibitor | TIU/mg | Raw 22.6 Meal 3.4 |
| Urease         | ∆pH     | Raw 2.18 Meal 0.03 |
| Isoflavones:   |         |                    |
| Total genistein| µg/g sample | 833 - 830 - 830 - |
| Total daizdein | "       | 734 - 721 -        |
| Total coumestrol | " (a) | 4.6 - 5.4 -       |
| Total biochanin | "       | 1.6 - 1.9 -       |

(a) Detection limit = 10 µg/g
(b) No significant difference is observed between parental and glyphosate resistant.

Insect damage and mycotoxin contamination of maize

Pest protected plants mainly concerned maize commonly used as fresh and whole plant in animal feeding and cotton only marginally used as cotton-seed meal in animal (ruminant) feeding (Faust, 2002). The protection of these crops against damage caused to the maize plant during the growing season can prevent the development of moulds from *Fusarium sp* in the maize cob as well as in the whole plant. Pre and post harvesting growing of moulds eased in damaged crops lead to the simultaneous production of numerous mycotoxins generally deleterious for the health of animals. Recent data collected on the level of fumonisin B1 in maize grown in different European countries such as Germany, Italy Spain and France (Cahagnier and Melcion, 2000) and in the USA (Munkvold et al., 2002) are interesting to discuss. Insect resistant (Bt) maize is in general efficiently protected from the damage caused by ear worms, leading to a spectacular reduction in the proportion of maize cobs contaminated by *Fusarium*. The kernel and the whole plant are subsequently less contaminated by Fumonisin B1 (Table 3). Additional data indicated that the level of fumonisin continue to increase even during storage of the silage although the level of contamination is apparently not linked with the level of moisture at harvesting.
stage. However, the level of contamination is highly dependent on the genotype and the sensitivity of the plant, the climatic conditions, and the development of pests (Cahagnier and Melcion, 2000). But no clear indications are available in the scientific literature on the in vivo effect of fumonisin B1 as that of deoxinivalenol which is always associated with fumonisin in the kernel (Valenta et al., 2001). Growth retardation in the piglets fed maize grain has been associated with the presence of both toxins in an isogenic control variety of maize used in the experiment (Piva et al., 2001a). These data indicated a potential advantage of pest protected GM maize in the prevention of mycotoxin development and the safety for farm animals of new maize protected from the European corn borer.

Safety assessment and nutritional equivalence of GM feeds for farm animals

The assessment of the safety of new feeds for farm animals and consequently of the safety of food from animal origin has lead to the recommendation expressed by international bodies or individual countries (OECD, 2000; Chesson, 2001; Kletter and Kuiper, 2002). It implies numerous molecular, biochemical, biological and toxicological methods which are individually seldom sufficient to conclude on a final assessment. The initial assessment of the eventual toxicity of inserted genes and expressed protein essayed by in vitro and/or in vivo methods or with the methods of acute toxicity will not be presented and discussed in the present paper (For more information, see Noteborn et al., 1994).

Only the assessment of the whole feed issued from GM pest protected plants (Bt) or herbicide tolerant to glyphosate and glufosinate will be developed. The safety for farm animals receiving a diet containing up to 95% of these plants in case of fattening steers and the safety of the food chain has been particularly considered. Results on experiments comparing GM plants to their isogenic counterparts published through September 30, 2003 in the specific international scientific literature have been used. They can be considered a comprehensive basis to assess the safety and the nutritional value of plants incorporated at the maximum level in balanced diets chronically fed to farm animals. The following procedure has been used to compile the data issued from the comparison of new plants to their near isogenic counterparts:

- The different parameters, depending on the animal species and measured during the experimental tests are used;
- The equivalence of the new feeds compared to the near isogenic control plants is established

| Genotype (G) | Plant (M) | A | B | Significant effects (c) |
|-------------|-----------|---|---|-------------------------|
|             | DM % of fresh plant (a) | 37.6 | 35.7 | 41.3 | 41.0 | G* |
|             | Fusarium ear rot % of cobs (b) | 29.2 | 10.6 | 41.9 | 10.2 | G*; M* |
| Fumonisin B1(µg/g fresh): | Kernel | 21.2 | 4.9 | 32.7 | 8.3 | G*; M* |
|             | Fresh plant | 4.1 | 2.9 | 6.2 | 1.2 | G*; M* |
|             | Silage | 6.6 | 6.0 | 6.0 | 5.0 | G*; M* |

(a) Dry matter content of plant at harvesting
(b) As % of damaged cobs on total cobs
(c) G* and M*: Significant effects of genotype and of pest resistance, respectively (P<0.05)
in the absence of significant difference between average results observed for the different treatments;

- Absolute values of performance of animals involved in the comparison of performance supported by GM plants are not given. Only the number of equivalent responses to the control feed will be recalled and expressed relatively to the total number of experiments performed on new feeds;

- Corresponding references are listed as footnotes to the tables.

Safety and comparative nutritional values of pest protected (Bt) and herbicide tolerant plants

The safety of the new plants obtained by genetic engineering must be demonstrated prior to the dissemination in the environment in Europe and in the U.S. (Council Directive EEC, 1990; Directive 2001/18/EC, 2001; US FDA 1992). Laboratory animals have been used in the first steps of the demonstration of the safety of new plants (Hammond et al., 1996). However, very few data have been published in the scientific literature on the safety and the nutritional value of GM plants in laboratory animals. Also, nutritionists have favored farm animals for which numerous studies have been completed and results published in reviews or abstract papers. Zootechnical data classically demonstrated the particularly high sensitivity of high producing dairy cows or fast growing chickens to the presence of either ANFs, toxic substances and the imbalance of their diet in available nutrients. All authors estimate that any deleterious effect caused by a diet based on new plants would point out defects or toxicity.

The safety and the nutritional properties of pest protected plants (Bt) are summarized in Tables 4 and 5. Thus, Bt maize kernels used as a basic ingredient of the diet of different animal species, never show an adverse effect on average weight gain, feed conversion, digestibility of nutrients and body composition during appropriate minimum duration lasting, for instance, the entire life of broilers. Experimental hazards occurred in two experiments performed with Bt MON810 and corn root worm protected event MON863 maize fed to broiler chickens and fattening steers, respectively. They even demonstrate in two cases a significant improvement (P< 0.05) of their perfor-

| Species and reference | Weight gain | Feed conversion ratio | Nutritional equivalence, digestibility | Body composition |
|-----------------------|-------------|-----------------------|---------------------------------------|------------------|
| Chicken:              |             |                       |                                       |                  |
| Bt Maize kernel (b)   | 7/8 (f)     | 9/9                   | 6/6                                   | 4/4 (a)          |
| Bt soybean (c)        | 1/1         | 1/1                   | 1/1                                   |                  |
| Pig (d)               | 5/5         | 5/5                   | 7/7                                   | 3/3              |
| Fattening steer (e)   | 1/1         | 0/1 (f)               | 1/1                                   | 1/1              |

(a) Including the weight of fat pad.
(b) Brake and Vlachos, 1998; Aulrich et al., 2001; Piva et al., 2001a; Gaines et al., 2001a; Taylor et al., 2001a; Sidhu et al., 2000; Taylor et al., 2001b.
(c) Kan et al., 2001.
(d) Weber et al., 2000; Aulrich et al., 2001; Piva et al., 2001a; Reuter et al., 2001; Gaines et al., 2001b; Fischer et al., 2003; Bressner et al., 2003.
(e) Van der Pol et al., 2002.
(f) Two experiments in which Bt MON 810 and MON 863 maize, respectively lead to a significant improvement of performance compared to the control (P< 0.05).
### Table 5. Safety and nutritional equivalence of pest protected (Bt) plants for ruminants. Values indicate the number of equivalent responses in the comparison between near isogenic parental and GM plants.

| Plant, reference | Animal    | Feed intake | Nutritive equivalence | Milk production | Milk composition | Weight gain | Body score |
|------------------|-----------|-------------|-----------------------|-----------------|------------------|-------------|------------|
| Maize silage (a) | Dairy cow | 7/7         | 5/5 (d)               | 7/7             | 7/7              | -           | -          |
| Maize silage (b) | Steer     | 4/4         | 3/3 (d)               | -               | -                | 4/4         | 4/4        |
| Cotton meal (c)  | Dairy cow | 3/3         | -                     | 3/3             | 3/3              | -           | 3/3        |

(a) Folmer et al., 2000; Faust, 2000; Barrière et al., 2001; Grant et al., 2003; Donkin et al., 2003.
(b) Aulrich et al., 2001; Maierhof et al., 2000; Hendrix et al., 2000; Berger et al., 2003.
(c) Castillo et al., 2001a; Castillo et al., 2001b.
(d) Digestibility trials on adult rams, or in vitro studies.

### Table 6. Safety and nutritional equivalence of herbicide tolerant plants (Gly= glyphosate; Glu= glufosinate). Values indicate the number of equivalent responses in the comparison between near isogenic parental and GM plants.

| Plant     | Animal    | Feed intake | Weight gain | Feed conversion | Carcass composition | Nutritive equivalence | Milk: Prod./Composition |
|-----------|-----------|-------------|-------------|-----------------|----------------------|-----------------------|-------------------------|
| Maize (Gly) (a) | Chicken | 4/4         | 4/4         | 4/4             | 2/2                  | 4/4                   | -                       |
|           | Pig       | 2/2         | 2/2         | 2/2             | 1/1                  | 3/3                   | -                       |
|           | Steer     | 3/3         | 3/3         | 3/3             | 3/3                  | 3/3                   | -                       |
|           | Dairy cow | 4/4         | -           | -               | -                    | -                     | 5/5                     |
| Soya (Gly) (b) | Chicken | 1/1         | 1/1         | 1/1             | 1/1                  | 1/1                   | -                       |
|           | Pig       | 1/1         | 1/1         | 1/1             | 1/1                  | -                     | -                       |
|           | Dairy cow | 1/1         | -           | -               | -                    | 1/1                   | 1/1                     |
| Cotton (Gly) (c) | Dairy cow | 2/2         | -           | -               | -                    | -                     | 2/2                     |
| Canola (Gly) (d) | Lamb    | 1/1         | 1/1         | 1/1             | 1/1                  | 1/1                   | -                       |
| Maize (Glu) | Pig       | -           | -           | -               | -                    | 1/1 (f)               | -                       |
| Beet (Glu) (e) | Pig      | -           | -           | -               | -                    | 1/1 (f)               | -                       |
|           | Ram       | -           | -           | -               | -                    | 1/1 (f)               | -                       |

(a) Chicken: Gaines et al., 2001a; Taylor et al., 2001a; Sidhu et al., 2000; Taylor et al., 2001b; Hammond et al., 1996. Pig: Stanisiewski et al., 2001; Fisher et al., 2002; Gaines et al., 2001b. Steers: Simon et al., 2002; Berger et al., 2002. Dairy cow: Donkin et al. 2000; Donkin et al., 2003; Ipharraguerre et al., 2002; Grant et al., 2002; Grant et al., 2003.
(b) Hammond et al (1996); Cromwell et al., (2002); Hammond et al., (1996).
(c) Castillo et al., 2001a; Castillo et al., 2001b.
(d) Stanford et al., 2002.
(e) Böhme et al., 2001; Weisberg et al., 2001.
(f) Measurements on adult ram.
mance in comparison with animals fed near isogenic maize (Table 4). Another experiment conducted on laying hens fed a diet based on Bt maize demonstrated similar performance for laying rate, feed conversion expressed as feed/kg eggs produced and similar energy content as control maize (Aulrich et al., 2001). Whole plants of different Bt maize containing the expressed protein and fed successively up to 216 days to ruminants had no deleterious effect on feed intake, nutritional equivalence and average daily gain on the one hand in steers, and on the other hand on milk production and composition and body score of high producing dairy cows (Table 5).

Similarly, feed issued from GM plants and their derivatives have been tested in farm animals in the case of plants tolerant to specific herbicides. The results of a total of 23 experiments conducted on broiler chickens, pigs, fattening steers, growing lambs or adult rams and dairy cows have been summarized in Table 6. The nutritional equivalence of glyphosate resistant maize grain or whole maize silage expressed as metabolizable energy corrected for zero nitrogen balance (MEN) in poultry or as feed conversion ratio or milk production in dairy cows, respectively has always been demonstrated. Soybean, cotton or canola resistant to glyphosate fed to appropriate animals as raw seeds or meals after oil extraction were also nutritionally equivalent to their parental counterpart. Similar comparative experiments have been performed with kernels of maize resistant to glufosinate fed to pigs and adult rams. Digestible trials also demonstrated nutritional equivalence between feed derived from modified and control plants (Table 6). Recently, an experiment conducted on swine

| Plant | Parental | Gly tolerant |
|-------|----------|--------------|
| Nitrogen balance, gN/cow/day: | | |
| Intake | 840 | 851 |
| Faeaces | 236 | 240 |
| Urinary | 446 | 467 |
| Milk | 236 | 240 |
| Ruminal metabolism: | | |
| N NH3, mol/L (a) | 0.111 | 0.116 |
| Acetate (b) | 70.7 | 70.9 |
| Propionate (b) | 20.7 | 20.6 |
| Butyrate (b) | 7.8 | 7.5 |

(a) In rumen fluid
(b) As % of total volatile fatty acids.

Table 7. The incorporation of glyphosate tolerant soybean in the diet of dairy cows has no effect on nitrogen and ruminal metabolism (Adapted from Hammond et al., 1996).

| Animal | Steer (a) | Pig (b) |
|--------|----------|--------|
| Plant | Parental | Gly tolerant | Parental | Gly tolerant |
| Backfat depth | cm | 0.99 | 1.04 | 2.31 | 2.36 |
| Marbling score | | 559 | 549 | - | - |
| Muscle Longissimus: | | |
| Area | cm² | 82.6 | 84.5 | 58.6 | 56.6 |
| Moisture (c) | | 71.4 | 71.7 | 72.7 | 72.5 |
| Lipids (c) | | 4.6 | 4.3 | 2.2 | 3.0 |
| Proteins (c) | | 24.0 | 23.7 | 23.8 | 23.5 |
| Liver abscess (d) | | 0.24 | 0.22 | - | - |

Non significant differences between treatments, within experiment.
(a) Berger et al., 2002; Fisher et al., 2002.
(c) Per cent of the fresh weight.
(d) Score from 0 to 3; measured on 196 animals in each treatment.
fed diets containing glyphosate tolerant wheat for 100 days have shown similar performance to that of pigs fed diets containing non-transgenic control wheat (Peterson et al., 2003).

**Potential physiological and metabolic effects**

The majority of experiments designed to evaluate the nutritional equivalence do not generally include all specific observations recommended in toxicological tests recommended for assessing the safety of feed additives, chemical components or drugs. The dose/response test in case of feed ingredients is unfeasible and leads to additional experimental difficulties. Thus, no data are generally available on the organ weight such as liver, kidneys, digestive and reproductive tracts of experimental animals at slaughter. In the case of fattening steers and slaughter pigs, only the carcass yield has been measured and remained not affected by feeding GM plants in their diets (Tables 6, 8 and 9).

However, data observed on rats fed herbicide tolerant soybean failed to demonstrate significant effects on organ weight including kidneys and testes (Hammond et al., 1996). Complementary physiological investigations have concerned the hormonal status of dairy cows fed fodder beets resistant to glyphosate supplying 20/22% of the dry matter intake for 12 weeks. The mitogenic activity of milk whey fraction and blood plasma measured on mammary epithelial cells has not been modified in animals fed GM plants. Similarly, the serum level of insulin, of bovine growth hormone (bGH) and insulin like growth factor binding protein (IGF BP3) and enterolactone have not been modified by using feeds developed from GM beets. Incidentally, all these parameters can be significantly modified by merely changing the botanical composition of the basal forage or silage fed to the dairy cows (Weisbjerg et al., 2001). Studies on the potential metabolic effect of soybean meal issued from glyphosate resistant modified plant, neither demonstrate differences on the nitrogen balance, nor on the proportion of volatile fatty acids in the ruminal fluid. The pH of the rumen and the level of ammonia have also not been modified by the origin of the beans (Hammond et al., 1996).

**Quality of animal products and safety of the food chain.**

**Quality of animal products**

Experiments conducted by nutritionists investigating the potential consequences of feeding GM crops to farm animals have also concerned the control of the quality of animal products and milk. Typical comparative data collected on carcasses of steers and pigs are summarized in Table 8. They show no effect, neither on back fat thickness, nor on marbling scores of meat in steers fed glyphosate NK603 maize grain almost their entire life. Furthermore, no difference in the main characteristics of the Longissimus muscle expressed by loin area and chemical composition have been demonstrated. Also, pathological lesions expressed by the very low percentage of liver abscess in fattening steers have not been significantly modified by feeding glyphosate resistant maize silage to steers. Similarly, the addition of glyphosate resistant soybean meal in the diet of growing finishing pigs had no effect on the average lean daily gain, and the back fat thickness of the animals at slaughter. Physical characteristics of pork chops expressed by cooking losses and sensory traits of meat expressed by juiciness, tenderness and flavor were not affected by feeding conventional or glyphosate resistant soybean meal (Cromwell et al., 2002, Table 9).

Neither the quantity of fat corrected milk, nor the quality of milk characterized by its fat or protein content, nor the proportion of casein in the total protein have been modified in milk of dairy cows receiving insect protected maize silage (Faust, 2000; Barrière et al., 2001; Clarke and Ipharraguerre, 2001, Table 10). Initial data on the absence of modification of the chemical composition of Bt resistant and herbicide tolerant plants is generally considered as a good predictor of the results concerning the quality of the products issued from animal fed GM plants.

**Food safety**

Biological evidence of the presence of foreign (M13) DNA ingested by mice reaching peripheral leukocytes and internal organs has raised the important question on the fate of recombinant DNA
(rDNA) in tissues of farm animals (Schubbert et al., 1997). Systematic tests have been essayed on the detection of the presence of rDNA but also animal DNA and chloroplastic DNA in milk, animal tissues and eggs and eventually in rumen bacteria. However, it is relevant to recall that a cow fed Bt resistant maize silage only ingests 600 mg of total DNA daily and that only 0.00042 % can originate from CryIA(b) DNA (Beever and Kemp, 2000). Moreover, highly degradable CryIA(b) is either in non detectable amounts or only at very low signals in insect protected maize silage according to Fearing et al., (1997) and Faust (2000), respectively. During silage CryIA(b) protein is highly degraded; however, DNA is fragmented but still detectable months after silage (Hupfer et al., 1999). This degradation during the fermentative process of silage is further limiting the risk of recovering the rDNA in animal products. Extended tests using specific polymerase chain reaction (PCR) for DNA amplification have been used to characterize CryIA(b) or Cpaepsps rDNA in feeds and animal products. Results of 4 experiments available in the scientific literature on Cpaepsps rDNA are summarized in Table 1. The presence of Cpaepsps rDNA can be detected and even quantified in a diet containing as little as 0.1 % of GM plant (Phipps et al., 2002). However, in all cases, neither positive signal has been identified in milk of dairy cows fed 12 successive weeks glyphosate tolerant soybean meal (Phipps et al., 2002), nor in muscle of broilers and pigs fed glyphosate resistant soybean, respectively (Khumnirdpetch et al., 2001; Jennings et al., 2003). These results agreed with data of Faust (2000) observed on 147 samples of cow milk from animals receiving either insect resistant maize from Bt176 or Bt11 events. An original experiment also demonstrates the persistence of rDNA from glyphosate tolerant canola during processing for oil extraction. But only fragments of rDNA have been detected in ruminal fluid incubated with GM canola. Conversely, absolutely no amplifiable rDNA inserted in canola is present in rumen bacteria (Alexander et al., 2002).

Results of 8 experiments concerning Bt rDNA are also available: 3 of them on lactating cows, 1 on fattening steers, and 4 on chickens. They demonstrate, in particular in 3 experiments out of 3 for cow milk, or in 6 tests out of 6, the absence of Bt rDNA in muscle, liver, kidneys and spleen of broilers and in eggs and feces of laying hens, and further in liver, kidneys and spleen of fattening bulls as in feces of dairy cows (Einspanier et al., 2001). The specificity of the identification techniques has extensively been checked using samples of milk spiked with original Bt rDNA, (Faust, 2000). However, chloroplastic (maize) DNA has been transiently recovered in chicken muscles, liver, kidneys and spleen of broilers (Aulrich and Flachowsky, 2001). Similarly, plant DNA fragments of 140bp have been identified in the liver, the Longissimus dorsi muscle and in the ovary of the pig up to 72 hours after the administration of maize (Reuter and Aulrich, 2002; Reuter and Aulrich, 2003). As a consequence, data seem to indicate that the presence of exogenous DNA in tissues originating from food corresponds to a normal situation, but its biological significance is not known.

Table 9. The sensory properties of the meat are not affected by feeding growing finishing pigs a diet based on glyphosate resistant soybean (Adapted from Cromwell et al., 2002).

| Plant Parental Glyphosate tolerant |
|-----------------------------------|
| Lean gain g/d | 341 | 337 |
| Backfat thickness cm | 2.45 | 2.20 |
| Cooking losses % of fresh | 31.97 | 30.28 |
| Sensory traits (a): |
| Juiciness | 5.52 | 5.58 |
| Tenderness | 5.91 | 6.10 |
| Flavor | 5.74 | 5.95 |

(a) Sensory traits are graded from 1 to 8; no significant difference.
Table 10. Safety and nutritional equivalence of feeds from GM plants: pest resistant (Bt) maize; glyphosate tolerant soybean meal for the dairy cow: estimation from milk composition.

| Plant Genotype | Maize silage Parental | Bt176 | Bt11 | Maize silage Parental | Bt176 | Bt11 | Soybean meal Parental | Gly tolerant |
|---------------|------------------------|-------|------|------------------------|-------|------|------------------------|------------|
| Milk kg/day   | 40.4                   | 39.5  | 38.2 | 31.5                   | 31.8  | 29.4 | 29.5                   |
| Composition (a): Fat | 3.41                   | 3.50  | 3.47 | 3.69                   | 3.68  | 3.55 | 3.61                   |
| Protein       | 2.72                   | 2.66  | 2.80 | 3.20                   | 3.18  | 3.25 | 3.24                   |
| Total solids  | 11.59                  | 11.63 | 11.84 | -                      | -     | 12.5 | 12.57                  |
| Reference     | Faust, 2000            | Barrière et al., 2001 | Clarke and Ipharraguerre, 2001 |

(a) As per cent of fresh milk.

Table 11. Absence of transgenic plant DNA (rDNA); presence of chloroplastic plant DNA in animal products.

| rDNA | Recombinant DNA in Feed | Chloroplastic DNA Feed |
|------|-------------------------|------------------------|
| Item | Feed        | Detection of rDNA*      | Milk | Tissues | Bacteria | Milk | Tissues |
| Cow  (a) | Soy.meal | Gly ++ | 00 | - | - | - | - |
| Cow  (b) | Soy.meal | Gly + | 00 | - | - | - | - |
| Rumen (c) | Canola | Gly ++ | - | - | 00 | - | - |
| Chick (d) | Soy.meal | Gly ++ | - | 00 | - | - | - |
| Pig  (e) | Soy.meal | Gly ++ | - | 00 | - | - | - |
| Cow  (f) | Maize | Bt 00 | 00 | - | - | (+) | Lympho + |
| Steer (f) | Maize | Bt 00 | - | 00 | - | - | ++ |
| Chick (f) | Maize | Bt ++ | - | 00 | - | - | - |
| Cow  (g) | Maize | Bt ++ | 00 | - | - | - | - |
| Chick (h) | Maize | Bt ++ | - | 00 | - | - | - |
| Cow  (i) | Maize | Bt + | 00 | 00 | - | - | - |
| Chick (i) | Maize | Bt ++ | - | 00 | - | - | - |
| Chick (j) | Maize | Bt ++ | - | 00 | - | - | ++ |

Response to PCR: 00 total absence; (+) faint signal; + - degraded in silage; + and ++ presence demonstrated; - not checked.

(a) Phipps et al., 2002; (b) Klotz and Einspanier, 1998; (c) Alexander et al., 2002; (d) Khumnidpetch et al., 2001; (e) Jennings et al., 2003; (f) Einspanier et al., 2001; (g) Phipps et al., 2001; (h) Glen, 2001; (i) Faust 2000; (j) Aulrich and Flachowsky, 2001.
Finally, specific proteins coded by CryIA (b) DNA in Bt176 and Bt11 cannot be found according to Faust (2000) in 67 and 80 samples of milk, respectively, investigated from experimental cows fed GM maize silage. Also Jennings et al., (2003) failed to find CP4EPSPS protein in the muscle of pigs fed glyphosate tolerant soybean meal.

Conclusions

Almost all the experiments considered in the present paper have been conducted in national institutes or university laboratories under good laboratory practices. The present analysis concerned data collected on farm animals consuming up to 95% of their dry matter intake as genetically modified insect protected or herbicide tolerant plants. Results published in referred reviews lead to the following conclusions:

a) No deleterious effect concerning the eventual toxicity of GM plants approved for dissemination for animal feeding have been described;

b) Data confirmed that genetic constructs used for creating insect resistant and herbicide tolerant plants previously tested by appropriate bio molecular methods are safe for animal feeds;

c) Scientific methods used for the assessment of the safety and recommendations for the dissemination of new plants including their massive use in animal feeding have been always appropriate to detect eventual defects on health of animals and performance;

d) The absence of rDNA either as whole gene or fragment in animal products, milk, meat and eggs has always been confirmed in the results of 12 publications out of 12 published in the literature and observed in several hundreds of samples.

Research concerning the modern techniques used in plant breeding, including genetic manipulation must be encouraged in order to assess the safety of feeds for animals and further to produce safe food for humans. Further steps would concern the selection of plants more balanced in their chemical composition, in comparison with the nutritional requirements of farm animals. Higher nutrient content and a better availability of these nutrients (protein, amino acids, fatty acids, minerals, trace elements and vitamins) and less anti-nutritional factors are expected by the nutritionists, in vivo experiments conducted on target animals appropriately selected according to the destination of the plants (ruminants, monogastric animals) can be strongly recommended to assess the safety of new feeds in order to prevent potential risks of the presence of ANFs or toxic undesirable factors.

Presently farm animals in Europe and elsewhere already have received the daily maize, canola, cotton and soybean meal from GM plants. Previous tests on target animals performed to assess their safety before dissemination in the environment are prerequisites for preventing potential risks for the health of animals and the consumer of animal products.

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