5 Discussion

5.1 Incidence of genetically modified soybeans and maize in Egypt

The first objective of the present work aimed to investigate and monitor the incidence of genetically modified soybeans and maize in Egypt. The methods applied in this study based on DNA analyses by polymerase chain reaction (PCR) according to Article 35 of the German Federal Foodstuffs Act (Anonymus, 2002). These techniques are specific, practical, reproducible and sensitive enough to detect up to 0.1% GMO in food and/or feedstuffs. Furthermore, all of the techniques mentioned are economically and can be applied in Egypt and other developing countries.

Anklam et al. (2002) reported that numerous analytical methods (qualitative and quantitative) for detection and determination of GM soybeans, maize and derived food products. The new analytical issues and challenges cannot be addressed the integration of conventional and new molecular tools for plant varieties developed by genetic manipulation, which will give rise to an increasingly wide range of GMO with multiple gene constructs.

Most of the new analytical methods used for detection and quantification of GMO depend upon real time PCR TaqMan assays, DNA-Chip or LightCycler technologies. All of those are very expensive techniques and need experience before application. As a result, Egypt and other developing countries have to select the economical, sensitive detection methods as the approach applied in this work. From the economical point of view, all techniques applied in this investigation by DNA analyses using low cost, sensitive PCR are more practical and economically, which enable the developing countries to monitor GMO on their market.

5.1.1 Soybeans

The primer pair GM03/GM04 is specific for the single copy lectin gene LE1 in soybeans and yields a PCR product of 118 bp size as mentioned by Meyer et al. (1996). It is

detectable in transgenic as well as in conventional soybeans (soybeans specific). By using this primer pair, all tested soybean samples gave positive results. These results revealed that the DNA was successfully isolated and the isolated DNA could be amplified during PCR without inhibition.

The primer pair p35s-f2/petu-r1 is specific for the genetic modification construct in Roundup Ready® soybean and amplifies a 172 bp segment as described by Wurz and Willmund (1997). The primer pair attaches to the CaMV35S promoter sequence and the petunia hybrid chloroplast transit-signal sequence (the new construct in RRS). The amplicon is only detected in transgenic samples and GMO containing CRM.

The local Egyptian breeds of soybean seeds (27 samples) and the Egyptian full fat soybean sample revealed negative results after PCR by using primer pair p35s-f2/petu-r1. In contrast, all 13 samples imported from Argentina and 5 out of the 10 samples imported from USA tested positive for Roundup Ready® soybean. Thus the locally Egyptian breeds did not contain any genetically modified material concerning Roundup Ready® soybeans.

Currently no available data concerning the monitoring of GM soybeans in Egypt. The obtained results indicate that the local varieties of soybeans are non Roundup Ready® soybean (RRS), while the imported soybeans were highly contaminated by RRS.

5.1.2 Maize

The primer pair Ivr1-F/Ivr1-R is specific for the maize *invertase* gene and flanks part of exon number 3 of this gene. It gives rise to a 226 bp amplicon (Ehlers et al., 1997). This product is detectable in transgenic, as well as in conventional maize (specific primer pair for maize plant).

For the specific identification of transgenic maize event Bt176 by PCR the primer pair Cry03/Cry04 was used. The resulting sequence of 211 bp size is amplified from a genomic region between two adjacent genetic elements in the GM construct, namely the

CDPK (Calcium Dependent Protein Kinase) promoter and the N-terminus of the synthetic *CryIA(b)* gene (Hupfer et al., 1998). This 211 bp amplicon appears only in transgenic maize samples, as well as in GMO containing CRM.

Bt11 maize transformed by the bacterial *PAT* gene which codes for the enzyme phosphinotricine N-acetyl transferase giving rise to the resistance of Bt11 maize to the herbicide phosphinotricine as documented by Anonymous (2002). Primer pair IVS2-2/PAT-B was used for the detection of the transition site from the intron IVS2 into the *PAT* gene in Bt11 maize line (Anonymous, 2002). Figure 8 illustrates the results obtained by using IVS2/PAT-B primer pair. Positive samples and the positive control (0.1% GMO-CRM) were shown amplicons at the expected DNA size (189 bp) after amplification.

Primer pair VW01/VW03 flanks the transition site from the genomic maize DNA into the CaMV-Promotor in MON810 maize. Thus representing an event specific detection system according to Anonymous (2002). Primer pair T25-F7/T25-R3 was used for the detection of the transition site between the CaMV-terminator into the *PAT* gene in T25 maize. Figures 9 and 10 show the results obtained for maize lines MON810 and T25 respectively. Positive samples as well as prepared 1% positive controls revealed amplicons of the expected size of 170 and 209 bp respectively, while the negative samples and negative control (CRM-containing 0% GMO) gave no amplification product after PCR.

For the detection of StarLink™ maize commercial kit was used. An amplicon of 133 bp is specific for the presence of DNA constructs from StarLink™ maize. Positive control as well as samples containing StarLink™ maize revealed amplicons at the expected DNA size (133 bp). It did not occurred in negative control or negative samples.

All DNA isolated from native maize varieties cultivated in Egypt (33 maize grain samples) amplified by using the maize specific primer pair (Ivr1-F/Ivr1-R). On the other hand, all of them were negative to all primers used in this study to detect the different

GM maize lines (Bt176, Bt11, T25, MON810 and StarLink™). These results established that the local Egyptian maize varieties were non-transgenic.

In contrast, all imported maize samples tested positive for GM maize lines. Twenty maize samples collected from Egyptian market, which were of USA origin From these collected 20 samples 16 contained Bt176, 17 Bt11, 12 MON810, 19 T25 and 9 StarLink™ maize. Furthermore, of the 7 maize samples imported from Argentina 4 contained Bt176 and MON810, 5 T25, 6 Bt11 and 2 StarLink™ (Table 8).

From the demonstrated results, it is very clear that all imported maize samples contained more than one GM construct. Four samples even contained a mixture of all the five GM constructs investigated of these, one sample was from Argentina and three were from USA. The pattern of the distribution of the GM maize constructs among the imported samples was considerably varied and indicated different lots taken from the sampling localities.

The results clearly show that imported maize and soybeans intended for animal or human nutrition in Egypt contained GM varieties to a high degree, including mixtures of several lines. In contrast, all local Egyptian varieties were free from GMO with exception of one maize gluten sample which produced in Egypt but which was made from imported seeds.

Since no quantitative analysis was carried out, the absolute percentage of each GM line in the samples was not determined. However, the primary aim of this investigation was to present an overview on the situation which existed in 2000 - 2001 using highly sensitive, reliable methods that are capable of detecting even trace amounts of GMO. The positive controls used in this study contained 0.1% GMO, which reflect the sensitivity of the detection methods used.

All GMO examined here have been approved in other countries and have passed the local safety evaluation. Nevertheless it cannot be excluded, that non-approved GM breeds may enter in the uncontrolled markets. In recent years the area planted with

genetically modified (GM) crops has increased worldwide. Between 1996 and 2002, it rose from 1.6×10^6 to more than 58×10^6 hectares and will be increased by the same way as reported by James (2003). Therefore inexpensive, qualitative and sensitive methods, as used in the present investigation, would be suited for monitoring programmes especially in Egypt and other developing countries.

The urgent need to monitor feed and food for the presence of GMO is underlined by the example of StarLink™ maize. This GM maize line, produced by Aventis Crop Inc., has been assessed only for animal feed and use exclusively in USA. Recently it entered the food chain unintentionally although it was suspected to be associated with allergenic conditions in humans, but in spite of intensive investigations no allergic reactions were noted or attributed to StarLink™ maize as recorded by FDA and CDC (2001).

In conclusion, all local Egyptian varieties of both, soybean and maize contained no transgenic material from the constructs investigated in this study. On the other hand, the imported varieties of both, soybeans and maize contained a number of GM constructs. Therefore Egypt and other importing countries need to monitor the imported foods and feeds, if they required labeling of such products, to protect the local breeds from contamination and to inform the public about the presence of GMO.

5.2 Nutritional value assessment of Bt-Maize (hybrid NX 6262-Bt176 maize)

Bt-maize is an insect resistant maize plant originated by genetic modification. In addition, Bt-maize was found by high percent in the maize samples collected from Egypt during 2000 - 2001. The second goal of this work is to investigate the nutritional and safety aspects of a new hybrid of Bt176 maize (hybrid NX 6262).

5.2.1 Substantial equivalence

FAO/WHO (2000) and EC (2003) recorded that the concept of substantial equivalence was developed as a first practical approach to the safety assessment of genetically modified foods and feeds. Moreover, the consultation agreed that substantial

equivalence should be seen as a key step in the safety assessment process. Furthermore, Schauzu (2000) concluded that the principle of substantial equivalence is a reasonable approach to identifying differences between novel foods and their traditional counterparts.

As recommended by many authorities as OECD (1993), WHO (1993), FAO (1996), ILSI (1996) and EC (1997) safety assessment requires an integrated and stepwise, case-by-case approach for any new varieties of GMO. WHO (1995), FAO (1996) and Hammond et al. (1996) concluded that the compositional analysis of any new GM varieties considered sufficiently sensitive to detect potential material differences for new GM lines that may be developed.

Compositional analyses for maize grains (NX 6262-Bt176) in comparison with non-GM counterpart as well as proximate composition of both finishing diets (isogenic and transgenic diet) are presented in Table 9. These results clearly show that the levels of the proximate components in the grains of Bt176 maize were comparable to those in the conventional maize grains. In addition, these values were similar to the range of conventional standard values recommended by DLG (1995) or NRC (1995).

Analyses of 17 different amino and fatty acids revealed no difference between the transgenic maize grains and the conventional counterpart. Furthermore, the amino and fatty acids tested in the diets did not influence by the varieties of maize used.

The obtained results have shown that the genetic modification of maize used in this study (hybrid NX 6262-Bt176) did not change its content from the main nutrients and did not influence the main composition of the whole diet. These results are in agreement with that recorded for Bt-maize by Reuter et al. (2002). Based on these data, it can be concluded that the insertion of the construct of *Cry1A(b)* gene did not change the main nutrient contents of the GM maize grains. The results are also in agreement with the results of previous studies to determine the nutritional values of breed varieties of Bt176 maize used as feeds for different farm animals (Flachowsky et al., 2000, Böhme et al., 2001 and Aeschbacher et al., 2002a, b).

In a similar study Aulrich et al. (2001) compared different breeds of transgenic Bt-maize with the corresponding non-transgenic lines. The results of the analyzed maize samples illustrated substantial equivalence in all investigated ingredients, such as crude nutrients, amino acids, fatty acids, minerals and non-starch polysaccharides.

5.2.2 Stability of maize DNA in the broiler diets

Amplification of maize specific DNA fragments from both maize lines and diets was detectable using the primer pair Ivr1-F/Ivr1-R. This primer pair is specific for the maize *invertase* gene give rise to a 226 bp amplicon as described before by Ehlers et al. (1997). The PCR products were detected in both, transgenic and conventional maize. This indicates that the DNA was successfully extracted and it was amplified during PCR. For the specific identification of transgenic maize event Bt176, the primer pair Cry03/Cry04 was used, resulting in an amplicon of 211 bp as described by Hupfer et al. (1998). These 211 bp DNA fragments appear only in transgenic maize samples and in the 0.1% CRM positive control used.

To exclude the possibility of cross contamination between the control and experimental diets during preparation and mixing, samples from both diets were also subjected to DNA extraction and PCR techniques using the same primer pairs as mentioned above. All samples from control and experimental diets have shown positive results with primer pair Ivr1-F/Ivr1-R. While the primer pair Cry03/Cry04 only revealed positive results with samples from the diet containing Bt176 maize, which confirms that there was no cross contamination between the control and experimental diets.

These results indicate that the maize DNA can be also detectable in the diets after preparation. Crushing of maize grains or mixing with other diet ingredients cannot affect maize DNA integrity. The obtained results confirm the observation of Forbes et al. (2000) that grinding of plant did not cause significant disruption of DNA. These results are also in agreement with the previously report published by Beever and Phipps (2001), in which they discussed the impact of feed processing, including grinding, milling, heating and steam pressure on plant DNA integrity. However, this report

concluded that heat (>95°C) and high pressure substantially disrupt plant DNA, but grinding has no effect (heated or pressure treated feeds minimize the exposure of animals to contact plant DNA). In other investigation Berger et al. (2003) studied the influence of processing of isogenic and transgenic rapeseed on DNA-degradation. Both rapeseed varieties were treated by four different ways during manufacture process. The results of this study demonstrated that the degradation of DNA depends on the processing conditions. Mechanical treatment has no influence on the degradability of DNA, while the processes of extraction and toasting were resulted in high fragmentation of plant DNA.

In the present investigation the used feeds (isogenic and transgenic) were in the form of mash and did not going any further processing steps, which could negatively influence the DNA integrity in the diets.

5.3 Broiler performance

Table 12 shows the results of broiler performance parameters measured. However, the diets were formulated to allow a high proportion of the tested maize (73.58% maize in both control and experimental diets), the results clearly show that there were no significant difference (P>0.05) detected concerning feed intake, body weight gain and other performance parameters for both, control and experimental groups.

The results of performance parameters obtained in the present investigation are in agreement with the trials summarized and published Chesson and Flachowsky (2003) concerning the use of transgenic plants in poultry nutrition. They reviewed that comparative feeding studies with broilers and layers in which conventional maize (50 to 78%) or soybeans (27%) were replaced in mixed feeds by transgenic varieties, have failed to show differences of any significance in performance as well as in production parameters. However, Piva et al. (2001) observed a higher significant live weight gain in the broiler fed diet contained insect resistant GM maize (MON810) compared to the control group fed diet contained the conventional maize line. They concluded that lower mycotoxin content in the GM maize compared to the conventional maize might positively influence the weight gain.

The results respecting feed intake, body weight gain, feed conversion and digestibility of dry matter during the period of nutritional evaluation between days 20 - 25 showed that there were no significantly different between the both group and all the performance parameters mentioned did not influenced by the variety of maize.

According to Chesson and Flachowsky (2003) about 40 feeding studies with GM feed ingredients with various animal species have been reported in the literatures. Those involving poultry have included various lines of insect resistant (Bt) maize and glyphosate-resistant maize and soybeans. In each case diets were formulated to allow a high proportion of the test material to be incorporated (50 to 78% maize or 27% soybean) and comparisons were made with parental or near isogenic lines. In each study, the chemical composition of the GM feed ingredient proved to be essentially indistinguishable from its conventional counterpart. Consequently, and not surprisingly, comparative feeding studies with broilers and layers also failed to show differences of any consequence in various production parameters monitored.

All results concerning feeding value and performance of broilers fed the examined GM maize in this study (NX 6262-Bt176) were nearly identical for the isogenic and the transgenic maize diets. The results of the present study are in agreement with the recent study performed by Taylor et al. (2003). Comparison study of broiler performance, when fed diet containing grain from different GM maize varieties (Roundup Ready maize or Roundup Ready maize mixed with insect resistant MON810 maize), non-transgenic or commercial maize was carried out. Final live weights and feed conversion were similar across all groups. Thigh and wing weights were not affected by diets as well. The Authors concluded that broilers performed consistently and had similar carcass yields and compositions when fed diets containing Roundup Ready maize or Roundup Ready maize mixed with insect resistant MON810 maize as compared with their respective non-transgenic control and commercial maize.

The results obtained in the present investigation are also in agreement with the recent data obtained by Halle et al. (2004). Feeding GM-maize (Bt176) to breeder quails through 4 generations. They concluded that there is no significant difference (from 1st to 4th

generation) between the group fed diet contained Bt176 maize and the control group reared on conventional maize concerning feed intake, laying intensity and hatchability percent.

Table 17 summarized some feeding studies with various GM feed included in different poultry diets to assess chemical composition and nutritional value to poultry of GM maize in comparison with conventional parental or near isogenic lines.

Table 17. Comparison of chemical composition and nutritional value to poultry of GM maize kernels with conventional parental or near isogenic lines

Author	Transgenic feed ingredient	Results of compositional analyses	Poultry categories	Results of nutritional assessment
Hammond et al. (1996)	RR-soybeans	No significant difference	Broilers	No significant difference
Brake and Vlachos (1998)	Bt176-maize	No significant difference	Broilers	Feed : gain ratio improved ($P<0.05$) in Bt group
Mireles et al. (2000)	Bt176 maize	No significant difference	Broilers	No significant difference
Sidhu et al. (2000)	RR-maize	No significant difference	Broilers	No significant difference
Aeschbacher et al. (2001)	Bt176-maize	No significant difference	Broilers	No significant difference
Aulrich et al. (2001)	Bt176-maize	No significant difference	Broilers and layers	No significant difference
Gaines et al. (2001)	MON810-maize	No significant difference	Broilers	No significant difference
Kan et al. (2001)	Bt-soybean	No significant difference	Broilers	No significant difference
Piva et al. (2001)	MON810 maize	No significant difference	Broilers	Higher live weight gain ($P<0.05$) in Bt group
Taylor et al. (2001a and b)	RR-maize	No significant difference	Broilers	No significant difference
Aeschbacher et al. (2002a)	Bt176-maize	No significant difference	Broilers	No significant difference
Taylor et al. (2003)	RR-maize	No significant difference	Broilers	No significant difference
Halle et al. (2004)	Bt176-maize	No significant difference	Breeder quails	No significant difference

5.4 Blood and serum enzymes investigation

Blood and serum enzymes are a useful, sensitive indicator of the bird's general health. The PCV is a simple test provides fast and general information about the general state of examined whole blood and the bone marrow response. In a normal, healthy chicken, the PCV ranged between 30 - 55% (Gylstorff and Grimm, 1998). The PCV in both, experimental and control group were within the normal physiological limits. The health conditions were coincided with the low values of GOT, GPT and uric acid in serum, which indicates that the liver and kidney were in a good functional state without suffering from any acute infection.

The normal growth of the bone as well as the activity of metabolic processes was verified by the normal physiological limit of serum alkaline phosphatase and γ-GT respectively. Serum value of γ-GT is a good mirror for the overall body enzymatic and metabolic processes. These results showed clearly that the general health and metabolic processes of the birds were not affected by the new variety of maize used, and the investigated parameters were in the normal average as mentioned by Gylstorff and Grimm (1998).

From these obtained results, it can be concluded that the genetic modification, which increases the tolerance of maize plants towards insects, has no significant influence on the general health and physiological processes of broiler chickens.

5.5 Maize DNA degradability in broiler gut

Tables 15 demonstrated the Cycle threshold (C_T) obtained using ZM1-F/ZM1-R/ZM1 primer-probe-system in digesta samples collected from both, control and experimental group after feed withdrawal. The ZM1-F/ZM1-R/ZM1 primer-probe-system is specific and highly sensitive to detect a part of the *high mobility group* gene in maize plant (Newly established for this study by GeneScan Analytics GmbH, Germany).

The *high mobility group* gene can be detected in both, isogenic and transgenic maize plant and revealed PCR products at very small DNA size (79 bp), which is suitable to detect fragments of maize genome after digestion and degradation.

The plots generated by real-time PCR represented the standardized ΔR_n value (normalized reporter dye fluorescence) as a function of the number of cycles. Cycle threshold (C_T) is inversely proportional to the number of template copies present in the reaction sample, therefore the higher the initial amount of genomic DNA tested, the sooner accumulated product is detected in the PCR process and the lower value of the C_T (Heid et al. 1996).

The relative concentration of digesta in the crop, proventriculus and gizzard was high when the feed offered continuously till slaughter. The empty of the crop is controlled by the movement of digesta down from the muscular part of the stomach. In the duodenum the concentrations of all ingesta slightly decreased, because the duodenum is the shortest and narrowest part of the intestinal tract and the time of passage of ingesta in the duodenum is only about 10 min as recorded by Shires et al. (1987). In addition, the action of different digestive enzymes including DNase and RNase attack digesta at the duodenum level. Furthermore, in poultry, there is a duodenum reflux, which transports the ingesta back to the stomach and this mechanism affect the duodenum content (Whittow, 2001).

Shires et al. (1987) studied the rate of passage of maize containing diets through the gastrointestinal tract of broiler and white leghorn chickens. The results of this study were summarized in Table 18.

Table 18. Mean retention time (min) using insoluble marker in broilers and laying hens GIT

GIT segment	Broilers	Leghorns
Crop	31	48
Proventriculus + Gizzard	39	71
Duodenum	10	7
Jejunum	84	85
Ileum	97	84
Caeca	119	112
Rectum	56	51

(according to Shires et al., 1987)

Transit time of digesta is influenced by genetics. When comparing broiler and Leghorn-type chickens using insoluble marker, the overall mean retention time is not different, but the time food spent in various parts of the digestive tract is different. The rate of food passage is affected by many factors. Sell et al. (1983) concluded that feed transit time through the GIT increases with age, adding lipid or proteins and fasting. Increases in the environmental temperature slow the transit time.

The results obtained in the present investigation revealed that the maize DNA take the same pattern of passage along broiler GIT as normal ingesta, whether it originates from isogenic or transgenic maize line.

At the jejunum and ileum levels, which are the sites of absorption, the relative concentrations of poorly or undigested materials are relatively increased (Whittow, 2001). The relative concentration of maize DNA in jejunum and ileum in both groups was relatively increased, which indicates that DNA resists digestion in broiler GIT and subsequently is poorly absorbed. The content in the caeca and rectum affected by dropping of the birds, which frequently occurred and subsequently affect the results obtained at these sections of the GIT.

Samples collected from GIT contents of the birds slaughtered at 24h after the last feeding (control and experimental) were also revealed positive results. However, the results obtained appear at high C_T values (low maize DNA content in the samples), which may indicate excretion or partially degradation of the target DNA. These results are confirmed with the results obtained from analyses of the faecal matter, which collected between 20 - 25 days and were also revealed positive results for detection of maize DNA.

From the obtained results it is clear that maize DNA can resist in the gastrointestinal tract of broiler chickens without much degradation or absorption and excreted via the faecal matter. In recent study Reuter and Aulrich (2003) concluded that feed-ingested DNA is partially resistant to the mechanical and enzymatic activities of the GIT and is not completely degraded.

These results confirm that the absorption of functional large size DNA is low. Depending on the obtained results, it can be concluded that there is no evidence to absorb large functional size gene (DNA) and there is no precedence for the foreign DNA being incorporated into host cells beyond the use of the basic nucleotide building blocks as nutrients especially in organs responsible for metabolism as liver and kidney and those will be disappear by time as a normal disposal mechanism. These finding are in agreement with previous results published by Doerfler et al. (1997) and Beever and Phipps (2001).

In the digesta samples collected from the experimental group after feed withdrawal, Bt-maize DNA was also amplified using Cry2-F/Cry2-R/BTSYN primer-probe-system. These results detected that the GM construct can be also detected in the digestive tract and it resist the mechanism of digestion comparable as DNA derived from isogenc maize. Furthermore, both, maize specific and Bt DNA specific were also detected in all faecal samples, which collected at 20 – 25 days of age, all tested samples gave positive results by using TaqMan PCR technology.

The Bt-maize DNA detected in the samples collected at 24h after feeding (Figure 19) demonstrated that the genetic constructs in the Bt-maize resist digestion in broiler gut and subsequently are not absorbed. These finding are also in agreement with that demonstrated by Reuter and Aulrich (2003). They found that Bt gene fragment was observed in pig GIT at various times up to 48h after the last feeding of the diet containing Bt maize.

5.6 Metabolic fate of maize DNA in broiler blood, tissues and organs

All investigated broiler blood and tissue samples revealed positive results with MY-F/MY-R/MY-probe. This primer-probe-system amplifies mammals and poultry chromosomally encoded *myostatin* gene as recorded by Laube et al. (2003). The obtained results confirmed that the DNA was successfully isolated from blood, tissues and different organs as well as the isolated DNA able to be amplified during the PCR without inhibition.

The same samples were examined for the maize specific *high mobility group* gene (ZM1-F/ZM1-R-ZM1), for the Bt specific construct (Cry2-F/Cry2-R-BTSYN) and for the plant *chloroplast* gene with the plant 2 primer pair.

In contrast to the results obtained using tissue specific primer-probe-system, all investigated blood and tissue samples gave negative results with both maize specific and Bt specific primer-probe-systems (No amplification product was detected).

However, in the blood, pectoral and thigh muscles, liver, spleen and kidney samples from both trial groups, the plant DNA fragments (*chloroplast* gene fragments) were successfully amplified in the samples collected at 0h and 4h after feed withdrawal.

Interestingly, no more plant DNA amplification showed in either group in the blood and tissue samples collected at 24h after the last feeding (No amplification after PCR).

Sanderson and Walker (1994) concluded that intestinal epithelial cells have unique salvage pathways for using free nucleotides, owing to their high rate of cell turnover. Any small polynucleotide DNA fragments that might enter the body would be phagocytized by mononuclear leukocytes and further degraded by cellular enzymes and nucleases in different tissues, which was confirmed by the disappearance of *chloroplast* gene fragments detected in the tissues of broiler chickens after feed withdrawal in the present investigation. These results are also in agreement with the reports as summarized by Doerfler (2000).

The gastrointestinal tract is constantly exposed to DNA that is released from partially or completely digested food, ingested microbes, and DNA from intestinal microflora. Ingested food is mechanically disrupted and the released DNA, although it is poorly digested, cleaved through acid hydrolysis and enzymatic digestion into small DNA fragments and eventually some of these fragments are converted to single nucleotides. Acid hydrolysis in the gastrointestinal tract is expected to depurinate most adensine and guanine nucleotides of the food DNA (Klinedinst and Drinkwater, 1992). The presence of various phosphatases and deaminases continue to destroy the structural integrity of any free DNA. The breakdown products of DNA are absorbed for using at the cellular level for synthetic processes as they may be found in blood and tissues (McAllan, 1980 and 1982). The nucleotides are typically deaminated before being rapidly absorbed. Once absorbed, they are further catabolized into nitrogenous bases, free bases and other metabolites including sugars and phosphates that are used in cellular biosynthetic pathways as recorded by Sonoda and Tatibana (1978).

DNA is an essential component of all living organisms and as such, is present in nearly all foods and feedstuffs. In biotech crops, the introduced transgenic DNA molecules which based on the same basic chemical components as the endogenous DNA (adenine, guanine, thymine, and cytosine). Therefore, the introduction of transgenic DNA into a plant does not introduce any new chemical entities to foods. Furthermore, the total DNA in food contributes less than 0.02% to the total dry matter of the food as reported by Watson and Thompson (1988). Similarly, the amount of transgenic DNA in plants

manipulated through biotechnology represents a small proportion of the total amount of DNA in a biotech plant (<0.0004% of the total plant DNA), (Beever and Kemp, 2000).

Moreover, the genetic sequence coding protein introduced in a plant by biotechnology is only functional when the complete DNA sequence (gene) is activated in the plant as a complete gene without any degradation as mentioned by Beever and Kemp (2000).

A recent publication describes experiments that directly tested whether extensive feeding of DNA to mice results in detectable expression of mRNA and protein in organs of the animals (Hohlweg and Derfler, 2001). Approximately 50 mg of DNA, which encoded the green fluorescent protein (GFP) were fed to 21 mice for 3 weeks, and in a separate experiment that involved feeding 50 mg of the pEGFP-C1-DNA per day to mice over eight generations. No GFP protein or mRNA expression was detectable in liver, spleen, blood or intestinal epithelia of animals. Also, fragments of the GFP gene were not detectable by PCR analysis of DNA isolated from spleen, liver or tail tip samples from either this 3-week feeding study or that extended to eight generations. In other study they used a gene therapy approach, with intramuscular injection into mice of the GFP gene. These gene therapy studies showed clearly that detectable expression of the GFP protein and mRNA only at the site of injection. Therefore, it can be concluded from these studies that gene constructs capable of functioning *in vivo* when administered via a gene therapy procedure (e.g. intramuscular injection) and do not lead to gene expression in somatic cells or detectable integration into the germline of animals when provided orally.

In a similar study to the present investigation Jennings et al. (2003) studied the metabolic fate of YieldGard maize (MON810) in chickens breast muscle tissues in comparison with the conventional maize line. Chickens fed for 42 d with a diet including 60% MON810 maize (experimental group) or conventional maize (control group). Breast muscle samples from 10 chickens fed GM maize used and 10 chickens fed conventional maize grain were collected for DNA and protein analyses. Tissues were collected at the time of slaughter and subjected to DNA and protein analyses using PCR and ELISA. Fragments of GM maize DNA were not detected in any breast meat

samples in both groups. Additionally, using the same muscle tissue samples, Cry1A(b) protein (from MON810 maize) was not detected by ELISA technique as well. The results of this experiment are in agreement with the results obtained in the present investigation, thus the absence of detectable levels of transgenic DNA fragments in the tissue samples are confirm that the incidence of absorption a functional large size DNA from feed is very low.

From these results, it can be concluded that, feed ingested DNA is partially resistant to the mechanical, chemical and enzymatic activities of the broiler gastrointestinal tract and is not completely degraded. Isogenic and transgenic maize (Bt176) DNA was comparable during feed passage in the broiler gastrointestinal tract. Small DNA fragments derived from plant feeds can pass the gut epithelium and enter blood, some organs and tissues of broiler chickens, which disappeared after 24h of feed withdrawal.

6 Conclusions

1. From the first section of this study, it can be concluded that all local Egyptian varieties of both, soybeans and maize contained no transgenic material from the constructs discussed in this study. On the other hand, the imported varieties of both, soybeans and maize contained a number of GM constructs. Monitoring of imported foods and feeds seems to be necessary to know GMO contamination.

2. Isogenic and transgenic (Bt) maize were substantially equivalent on the base of compositional analysis data and performance of broilers.

3. Furthermore, the results showed that feed ingested DNA is partially resistant to the mechanical, chemical and enzymatic activities of the broiler gastrointestinal tract and is not completely degraded. Isogenic and transgenic maize (Bt176) DNA were comparable during feed passage in the broiler gastrointestinal tract. Small DNA fragments (199 bp) derived from plant feeds can pass the gut epithelium and enter blood, some organs and tissues of broiler chickens, which disappeared after 24h of feed withdrawal. Bt gene specific constructs from Bt176 maize could not be detected in any investigated blood or tissue samples.