4 Results

4.1 Detection and monitoring of genetically modified soybeans in Egypt

4.1.1 Detection of soybean DNA

The primer pair GM03/GM04 is specific for the single copy lectin gene LE1 and yields a PCR product of 118 bp size (Meyer et al., 1996). It is detectable in transgenic as well as in conventional soybean (soybeans specific primer pair) (Figure 4).

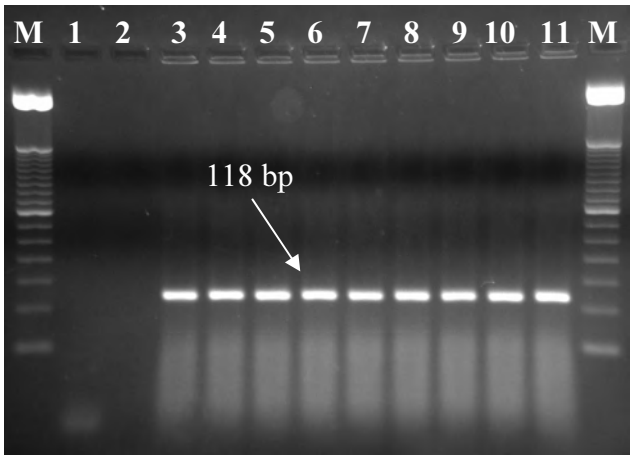

Figure 4. Detection of *Lectin* gene in soybean samples
(Results of PCR products of primer pair GM03/GM04)
M: 50 bp marker DNA, 1: blank sample (Extraction control), 2: PCR control, 3 and 4: local breed soybean seeds, 5 and 6: soybean meal (44% cp) imported from USA, 7 and 8: soybean seeds imported from USA, 9: CRM negative control, 10: CRM (5% GMO), 11: CRM (0.1% GMO)

Soybean specific primer pairs served as control for the amplification of isolated DNA and PCR procedures (PCR quality control).

4.1.2 Detection of Roundup Ready® soybeans

The primer pair p35s-f2/petu-r1 is specific for the genetic modification in Roundup Ready® soybean and amplifies a 172 bp segment (Wurz and Willmund, 1997). The

primer pair attaches to the CaMV35S promoter sequence and the petunia hybrid chloroplast transit-signal sequence. The amplicon is only detected in transgenic samples and GMO containing CRM as presented in the example in Figure 5.

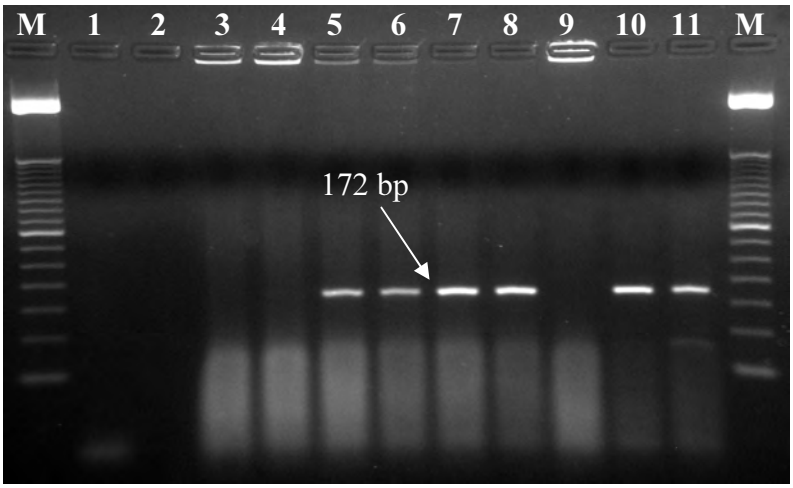

Figure 5. Detection of Roundup Ready™ soybeans
(Results of PCR products of primer pair P35s-f2/petu-r1)
M: 50 bp marker DNA, 1: blank sample (Extraction control), 2: PCR control, 3 and 4: local breed soybean seeds, 5 and 6: soybean meal (44% cp) imported from USA, 7 and 8: soybean seeds imported from USA, 9: CRM negative control, 10: CRM (5% GMO), 11: CRM (0.1% GMO)

4.1.3 Investigated soybean samples

Table 7 demonstrates the results of the examined soybean samples, its origin and the locality of collection from Egypt.

All 27 locally Egyptian breeds and the Egyptian full fat soybean sample tested negative in PCR analyses when 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^{TM} soybean. Thus the locally Egyptian breeds did not contain any genetically modified material concerning Roundup Ready™ soybeans.

Table 7. Results of examined soybean samples and its origin

Sample	Collection Locality[1]	Origin	GMO detection
27 Soybean seed samples of different cross local breeding	Giza	Local breed / Egypt	-
Soybean full fat	Giza	Local breed	-
Soybean meal	El-Gharbia	USA	-
Soybean meal	Giza	Argentina	+
Soybean meal	Bany-swef	USA	+
Soybean meal	El-Gharbia	Argentina	+
Soybean meal	El-Menia	Argentina	+
Soybean meal	Kalubea	Argentina	+
Soybean meal	Cairo	Argentina	+
Soybean meal	Cairo	USA	+
Soybean meal	El-Behara	USA	-
Soybean meal	El-Sharkea	Argentina	+
Soybean meal	El-Monofia	Argentina	+
Soybean meal	Alexandria	Argentina	+
Soybean meal	Kafr El-Shek	Argentina	+
Soybean meal	Ismailia and Port-Said	Argentina	+
Soybean seeds	El-Dakahlia	USA	-
Soybean meal	El-Dakahlia	Argentina	+
Soybean meal[2]	Cairo	USA	-
Soybean meal[2]	Giza	USA	-
Soybean meal	Giza	USA	+
Soybean meal	Asuit	Argentina	+
Soybean meal	El-Monofia	USA	+
Soybean meal	Wady El-Netron	Argentina	+
Soybean meal	El-Sadaat	USA	+

1) locality according to Governments in Egypt

2) soybean meal containing 48% crude protein

4.2 Detection and monitoring of genetically modified maize in Egypt

4.2.1 Detection of maize DNA

The primer pair Ivr1-F/Ivr1-R is specific for the 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 (maize specific primer pair), (Figure 6).

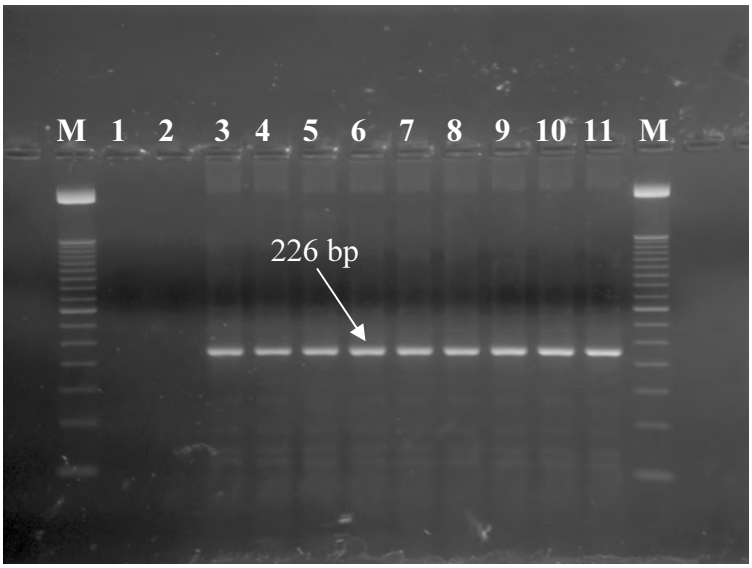

Figure 6. Detection of maize *invertase* gene
(Results of PCR products of primer pair Ivr1-F/Ivr1-R)
M: 50 bp marker DNA, 1: blank sample (Extraction control), 2: PCR control, 3 and 4: imported maize gluten from USA, 5 and 6: local breed of white maize, 7 and 8: yellow maize grain imported from USA, 9: CRM negative control, 10: CRM (0.1% GMO), 11: CRM (5% GMO)

Maize specific primer pair served as a control for the amplification of the isolated DNA and PCR procedure (PCR quality control).

4.2.2 Detection of GM maize lines

4.2.2.1 Bt176 maize

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, namely the Calcium Dependent Protein Kinase (CDPK) 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 GMO containing CRM (Figure 7).

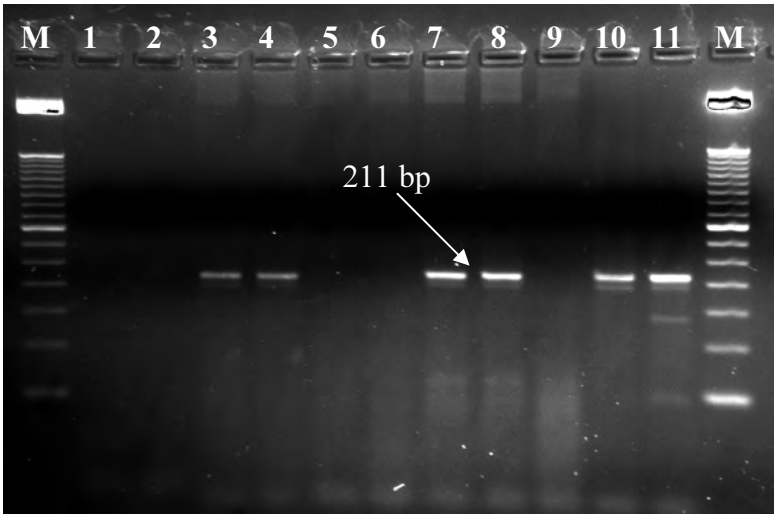

Figure 7. Detection of Bt176 maize
(Results of PCR products of primer pair Cry03/Cry04)
M: 50 bp marker DNA, 1: blank sample (Extraction control), 2: PCR control, 3 and 4: imported maize gluten from USA, 5 and 6: local breed of white maize, 7 and 8: yellow maize grain imported from USA, 9: CRM negative control, 10: CRM (0.1% GMO), 11: CRM (5% GMO)

4.2.2.2 Bt11 maize

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) show amplicons at the expected DNA size (189 bp).

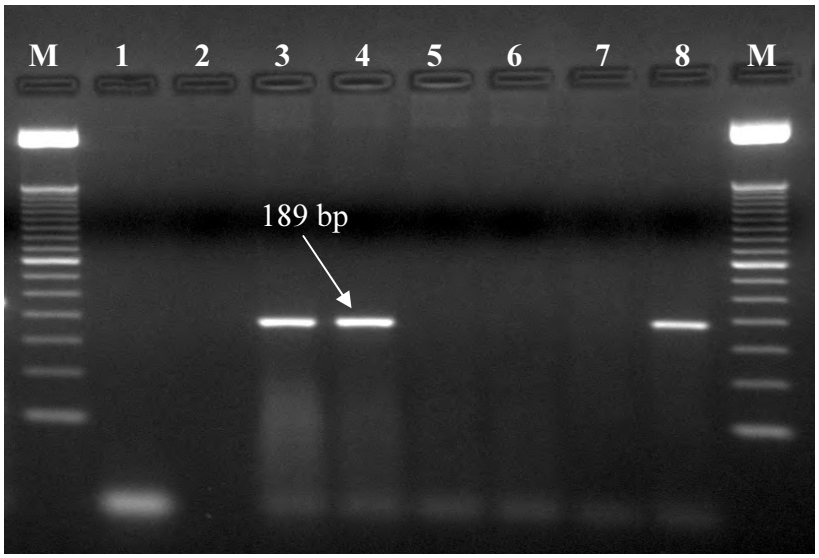

Figure 8. Detection of Bt11 maize
(Results of PCR products of primer pair IVS2-2/PAT-B)
M: 50 bp marker DNA, 1: blank sample (Extraction control), 2: PCR control, 3 and 4: imported maize grain from USA, 5 and 6: imported maize grain from Argentina, 7: CRM negative control, 8: CRM (0.1% GMO)

4.2.2.3 MON810 maize

Primer pair VW01/VW03 was used to detect MON810 maize line which amplify a fragment of 170 bp (Figure 9). Positive samples as well as 1% prepared positive control were positive, while negative control and negative samples revealed negative result (Anonymous, 2002).

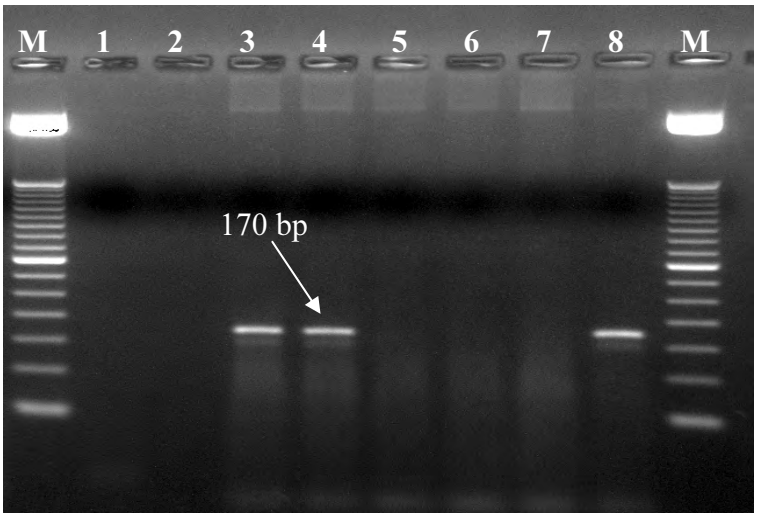

Figure 9. Detection of MON810 maize
(Results of PCR products of primer pair VW01/VW03)
M: 50 bp marker DNA, 1: blank sample (Extraction control), 2: PCR control,
3 and 4: imported maize grain from USA, 5 and 6: imported maize grain
from Argentina, 7: negative control, 8: prepared 1% GMO

4.2.2.4 T25 maize

Primer pair T25-F7/T25-R3 was used for the detection of T25 maize line (Anonymous, 2002). Figure 10 shows the results obtained for maize line T25. Positive samples as well as 1% prepared positive control revealed amplicons of the expected size of 209 bp. The negative control (CRM-containing no GMO) and negative samples gave no amplification product after PCR.

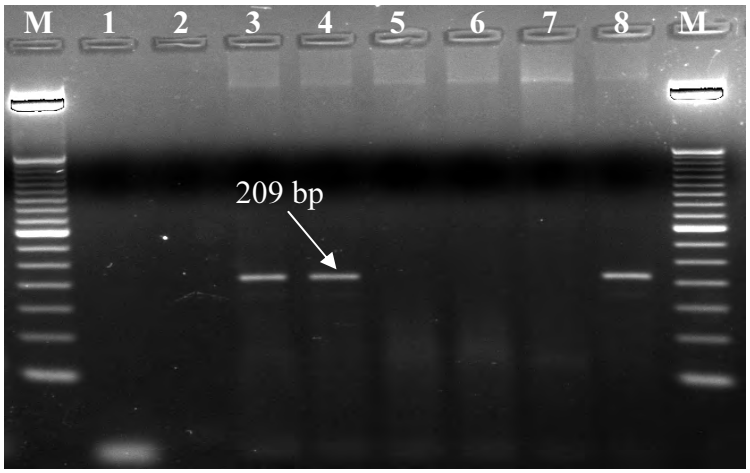

Figure 10. Detection of T25 maize
(Results of PCR products of primer pair T25-F7/T25-R3)
M: 50 bp marker DNA, 1: blank sample (Extraction control), 2: PCR control, 3 and 4: imported maize grain from USA, 5 and 6: local white maize grain, 7: negative control, 8: Prepared 1% GMO

4.2.2.5 StarLink™ maize

For the detection of StarLink™ maize commercial kit was used (GMO/Ident Kit StarLink^{TM} maize, Freiburg, Germany). An amplicon of 133 bp is specific for StarLink™ maize line (Figure 11). Positive samples as well as provided positive control revealed amplicons of at 133 bp DNA size. The negative control (provided with the commercial kit used) and negative samples did not amplified.

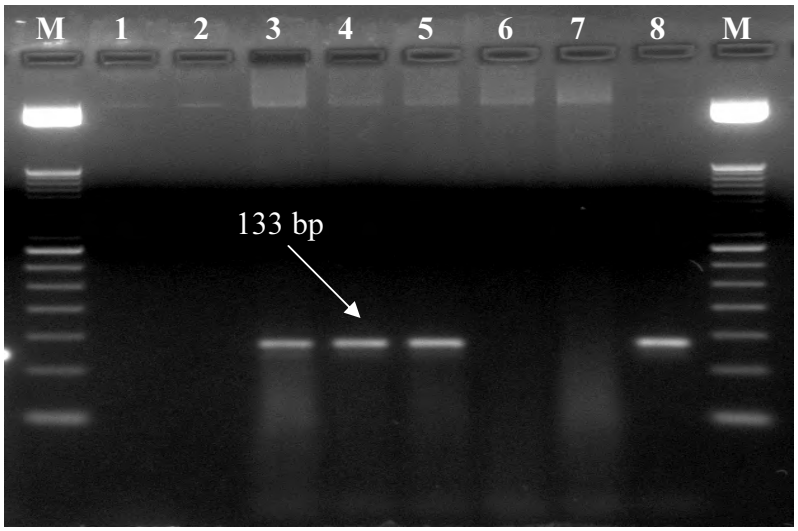

Figure 11. Detection of StarLink™ maize
(Results of PCR products of StarLink kit®)
M: 50 bp marker DNA, 1: blank sample (Extraction control), 2: PCR control,
3: imported maize grain from USA, 4: imported maize grain from Argentina,
5: imported maize grain from USA, 6: local Egyptian maize grain, 7:
negative control, 8: positive control
(negative and positive controls provided with the kit used)

4.2.3 Investigated maize samples

Table 8 demonstrates the results of PCR for the maize samples examined as well as its origin and collection locality.

All DNA extracted from native 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™).

With respect to the 20 maize samples imported from USA, 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™ (Figure 12).

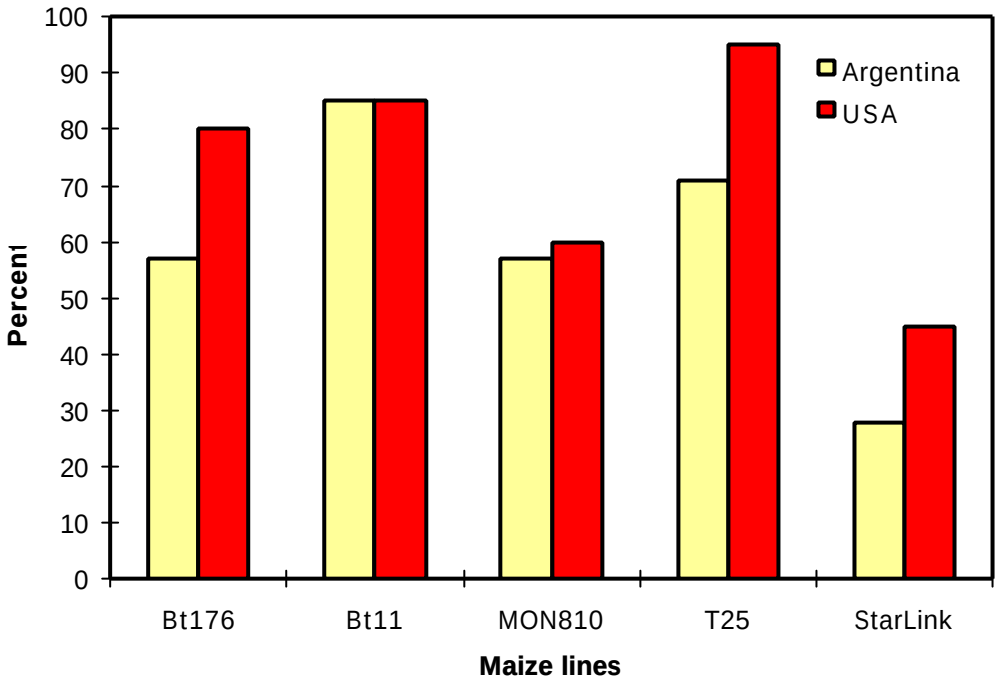

Figure 12. Percent of transgenic maize lines in the imported maize to Egypt (samples collected during 2000 - 2001)

Nearly all 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 sampling localities.

Table 8. Results of examined maize samples and its origin

Sample	Collection locality[1]	Origin	GMO detection				
			Bt 176 Maize (Cry03/Cry04)	Bt 11 Maize (Ivs2-2/PAT-B)	Mon 810 Maize (VW 01/VW 03)	T25 Maize (T25-F7/T25-R3)	StarLink Maize Test Kit
31 maize sample of different cross breeding	Giza	Local breed / Egypt	-	-	-	-	-
Local Maize grain (white maize)	El-Monofia, Luxor and Sohag	Egypt	-	-	-	-	-
Local Maize grain (yellow maize)	Asuit, Luxor and Kiena	Egypt	-	-	-	-	-
Maize grain	Giza	USA	+	+	+	+	+
Maize grain	Cairo	USA	+	+	+	+	-
Maize grain	El-Gharbia	USA	+	-	+	-	-
Maize grain	Bany-Swef	USA	+	+	+	+	-
Maize grain	Giza	Argentina	-	+	+	+	+
Maize grain	Cairo	Argentina	+	+	+	+	+
Maize grain	El-Menia	USA	+	+	+	+	+
Maize grain	El-Gharbia	Argentina	-	+	+	+	-
Maize grain	El-Behara	USA	+	+	+	+	-
Maize grain	El-Monofia	USA	+	+	+	+	+
Maize grain	Kalubea	Argentina	+	-	+	-	-
Maize grain	Alexandria	USA	+	+	-	+	+
Maize grain	Alexandria	Argentina	-	+	-	+	-
Maize grain	Kalubea	USA	+	+	-	+	-
Maize grain	El-Sharkea	USA	+	+	-	+	-
Maize grain	Ismailia a. Port-Said	USA	+	-	-	+	-
Maize grain	El-Dakahlia	USA	+	+	-	+	-
Maize grain	Kafr El-Shek	Argentina	+	+	-	+	-
Maize grain	Asuit	USA	+	+	-	+	-
Maize grain	El-Dakahlia	Argentina	+	-	-	-	-
Maize grain	El-Fayum	USA	+	+	-	+	+
Maize grain	Wady El-Netron	USA	+	+	-	+	+
Maize grain	Asuit	USA	+	+	+	+	+
Maize grain	El-Sadaat	USA	-	+	+	-	-
Maize gluten	Cairo	USA	-	-	-	+	-
Maize gluten	Wady El-Netron	USA	-	+	+	+	+
Maize gluten[2]	Wady El-Netron	Egypt	-	+	+	+	-
Maize gluten	El-Gharbia	USA	-	+	+	+	+

1) locality according to Governments in Egypt 2) maize grain imported from USA

4.3 Nutritional value assessment of Bt176 maize and derived feed

4.3.1 Proximate composition

Compositional analyses for maize grains and both finishing diets (isogenic diet contained the isogenic maize and transgenic diet contained transgenic maize) 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).

Table 9. Proximate composition of maize grains and diets [% DM]

Parameter	**Maize**		**Diet**	
	Isogenic	**Transgenic**	**Isogenic**	**Transgenic**
Dry matter (% fresh matter)	88.63	89.36	88.74	88.91
Organic matter (OM)	98.70	98.64	93.90	93.87
Crude protein (CP)	10.84	10.86	23.62	24.02
Ether extract (EE)	2.93	2.98	6.54	6.62
Crude fibre (CF)	2.33	2.54	1.72	1.67
Acid detergent fibre (ADF)	3.29	3.65	2.47	2.62
Neutral detergent fibre (NDF)	10.20	11.32	7.64	8.25
Total ash	1.30	1.36	6.10	6.13
Nitrogen free extracts (NFE)	82.60	82.26	62.02	61.56
Calcium (Ca)	0.20	0.26	1.44	1.46
Total phosphorous (P)	2.90	3.06	8.93	9.35
Potassium (K)	3.31	3.72	2.95	2.94
Starch	72.15	71.59	54.22	53.10
Sugars	1.66	1.82	1.51	1.65

4.3.2 Amino and fatty acids composition of maize grains and diets

The content of the 17 amino acids (Table 10) and the 17 fatty acids (Table 11) tested in the grains of Bt176 maize were comparable to those in the grains of the control line. All values are within the range determined for commercial varieties evaluated and published before (NRC, 1995).

Table 10. Amino acids composition of maize grains and diets [% DM]

Amino acid	**Maize**		**Diet**	
	Isogenic	**Transgenic**	**Isogenic**	**Transgenic**
Alanine	0.91	0.90	1.11	1.05
Arginine	0.46	0.45	0.85	0.84
Aspartic acid	0.77	0.79	1.17	1.15
Cystine	0.25	0.24	0.5	0.5
Glutamic acid	2.52	2.62	7.41	7.21
Glycine	0.40	0.38	0.85	0.82
Histidine	0.36	0.36	0.63	0.61
Isoleucine	0.43	0.45	0.87	0.9
Leucine	1.65	1.74	2.31	2.23
Lysine	0.28	0.27	1.33	1.26
Methionine	0.20	0.20	0.48	0.48
Phenylalanine	0.63	0.66	1.25	1.22
Proline	0.99	1.10	2.72	2.58
Serine	0.60	0.60	1.18	1.14
Threonine	0.44	0.43	1.07	1.05
Tyrosine	0.53	0.56	0.87	0.88
Valine	0.58	0.59	1.00	1.01

Table 11. Fatty acids composition of maize grains and diets [% EE]

Fatty acid	Abbreviation	Maize		Diet	
		Isogenic	Transgenic	Isogenic	Transgenic
Caprinic	C 10:0	0.04	0.04	0.03	0.03
Laurinic	C 12:0	1.21	1.16	1.12	1.17
Myristic	C 14:0	0.04	0.05	0.48	0.5
Palmitic	C 16:0	12.26	12.21	12.51	12.39
Palmitoleinic	C 16:1	0.19	0.18	0.64	0.65
Stearic	C 18:0	2.25	2.14	2.84	2.81
Olic	C 18:1	32.5	31.72	29.2	28.6
Linolic	C 18:2	47.68	47.32	47.4	47.4
Linoleic	C 18:3	1.12	1.15	2.83	2.82
Eicosanic	C 20:0	0.91	0.99	0.50	0.50
Eicosenic	C 20:1	0.2	0.2	0.17	0.18
Eicosadienic	C 20:2	0.02	0.02	0.02	0.03
Eicosatrienic	C 20:3	0.14	0.16	0.07	0.07
Eicosatetraenic	C 20:4	0.02	0.02	0.02	0.02
Behenic	C 22:0	0.23	0.24	0.37	0.37
Erucasic	C 22:1	0.24	0.22	0.32	0.31
Lignocerinic	C 24:0	0.19	0.17	0.18	0.16

Figure 13 and 14 demonstrate the similarity in percent of tested amino and fatty acids respectively between isogenic and transgenic maize. These obtained results clearly demonstrate that maize line Bt176 used in this study is comparable to the control line in respect to all parameters analyzed.

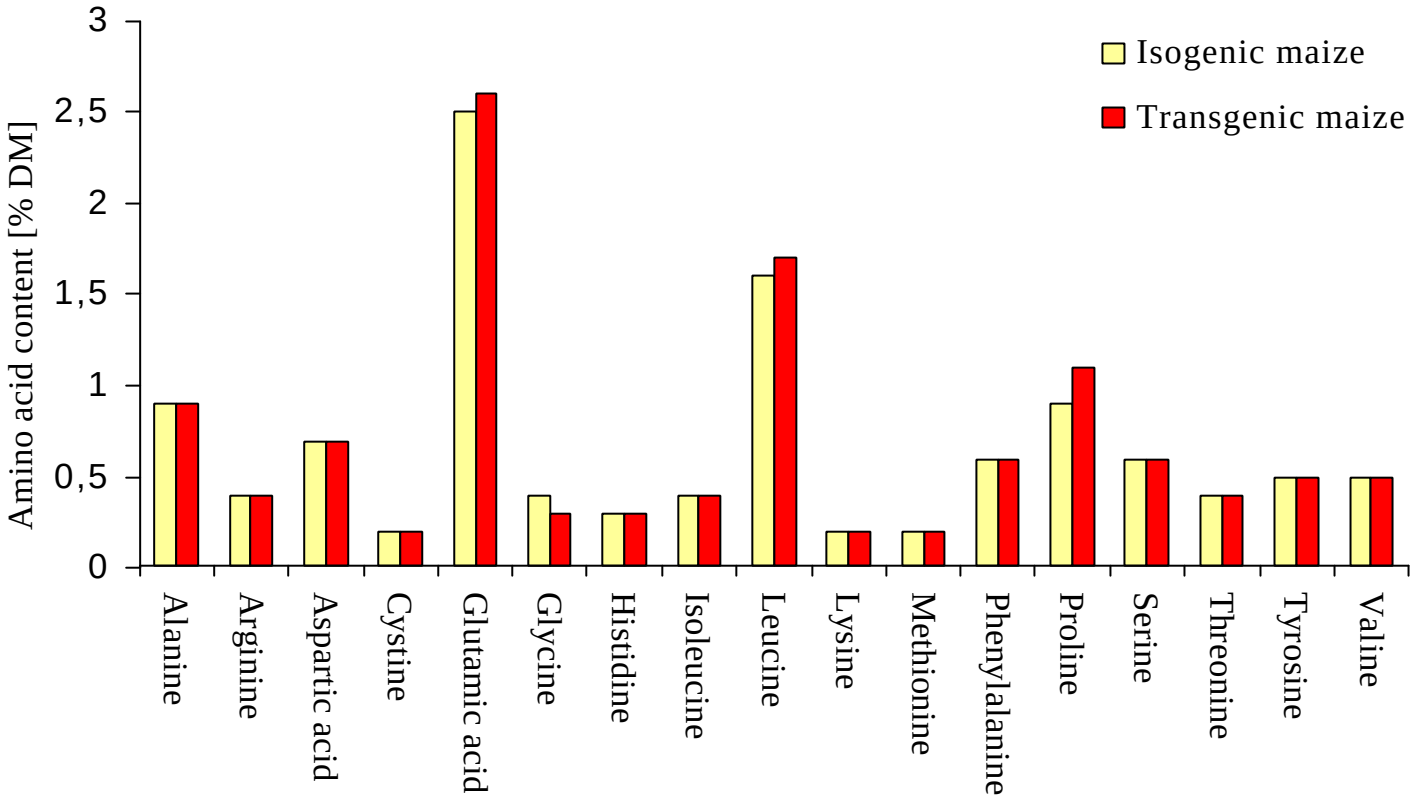

Figure 13. Amino acids composition of maize grains

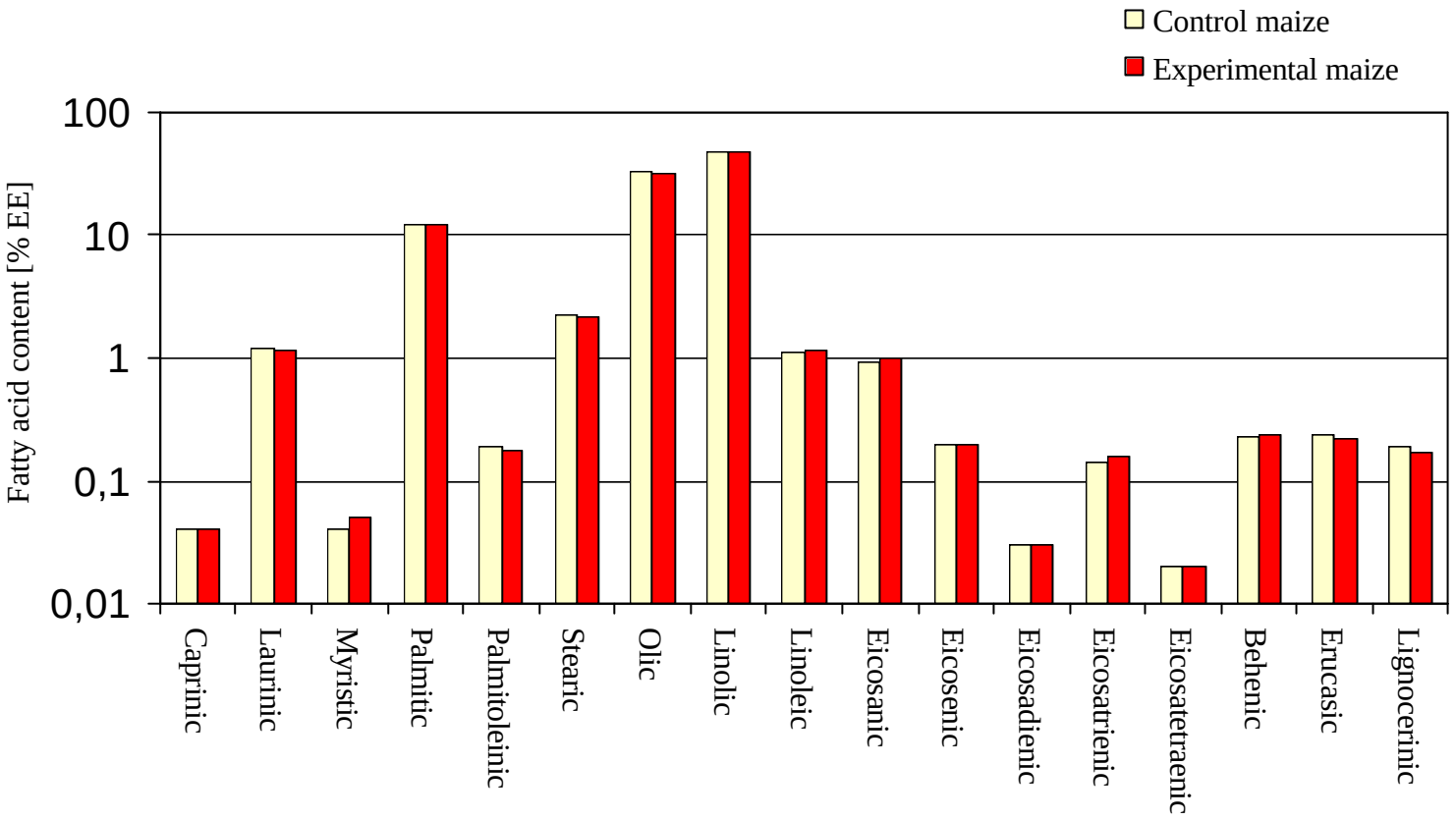

Figure 14. Fatty acids composition of maize grains

4.4 DNA Investigation in maize grains and 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 give rise to a 226 bp amplicon as described before by Ehlers et al. (1997). The PCR products were detected in both, transgenic and isogenic maize. This indicates that the DNA was successfully extracted and it was amplified during PCR (Figure 15A). For the specific identification of transgenic maize event Bt176, the primer pair Cry03/Cry04 was used, resulting in an amplicon of 211 bp (Hupfer et al., 1998). These 211 bp DNA fragments appear only in transgenic maize samples and in the 0.1% positive control used (Figure 15B), which demonstrate the specificity and sensitivity of the used PCR.

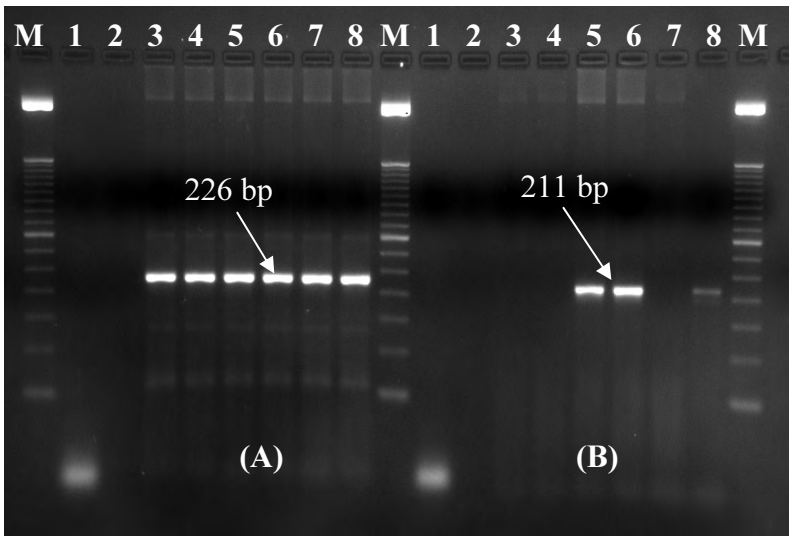

Figure 15 (A and B). PCR assays of investigated maize samples

(A) PCR products of mize specific primer pair & **(B)** PCR products of Bt176 primer pair

M: 50 bp DNA marker, 1: blank sample (extraction control), 2: water sample, 3 and 4: conventional maize, 5 and 6: Bt 176 maize, 7: negative control (0% GMO), 8: positive control (0.1% GMO)

To exclude the possibility of DNA 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. The results gave a similar results, which indicated that there were no cross contamination (Figure 16A and B).

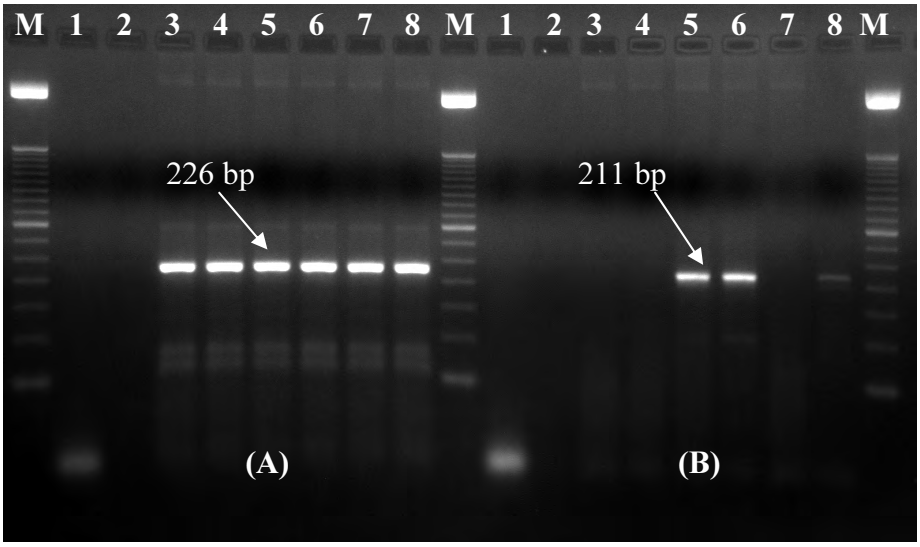

Figure 16 (A and B). PCR assays of investigated broiler diets

(A) PCR products of maize specific primer pair & **(B)** PCR products of Bt176 primer pair

M: 50 bp DNA marker, 1: blank sample (extraction control), 2: water sample, 3 and 4: control diet, 5 and 6: experimental diet, 7: negative control (0% GMO), 8: positive control (0.1% GMO)

4.5 Nutritional safety of Bt176 maize (Broiler performance, degradation and metabolic fat of DNA)

4.5.1 Broiler performance

The results of feed intake, body weight gain and other performance parameters for both, control and experimental groups are shown in Table 12

The results of this study had clearly shown 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 (see appendix Tables 10.2 - 10.6).

Table 12. Performance parameters measured (1-35 days)

Parameter	Unit	Control group	Experimental group
Feed intake	g	2190.7 ± 299.5	2096.1 ± 252.8
Body weight	g	1305.8 ± 192.9	1267.8 ± 166.6
Body weight gain	g	1263.5 ± 192.3	1225.8 ± 166.2
Daily weight gain	g	36.1 ± 5.9	35.0 ± 4.5
Feed conversion ratio	kg/kg	1.74	1.71

All treatments not significantly different (P>0.05)

4.5.2 Apparent digestible dry matter

The results of the apparent digestible dry matter, body weight gain, feed intake and feed conversion during the period of nutritional evaluation between days 20 - 25 are listed in Table 13. All demonstrated results were nearly identical for the non-transgenic and the transgenic diets. The values were not significantly influenced by the maize variety (P>0.05), (see appendix Table 10.7).

Table 13. Nutritional evaluation during the period of excreta collection (days 20-25)

	Control group	Experimental group
Feed intake (g/bird)	260.1 ± 25.9	251.7 ± 38.7
Body weight gain (g/bird)	150.8 ± 27.7	151.1 ± 39.7
Feed conversion ratio "FCR", (kg/kg)	1.72	1.67
Dry matter digestibility (%)	71.5	73.3

All treatments not significantly different (P>0.05)

4.5.3 Blood and serum enzymes investigation

Table 14 shows the results of packed cell volume (PCV) and some serum enzymes analyses. The PCV in both, experimental and control group are within the normal physiological limits. The health conditions were coincided with analyses of Glutamic oxalic transaminase (GOT), Glutamic pyruvic transaminase (GPT) and uric acid in serum. The normal growth of the bone as well as the activity of metabolic processes was verified by serum alkaline phosphatase and Gamma glutamyl transferase (γ-GT) respectively.

Table 14. Packed cell volume and some serum enzyme values

Parameter	Control group	Experimental group
Packed cell volume (PCV) [%]	32.3 ± 4.8	33.0 ± 4.5
Glutamic oxalic transaminase (GOT) [U/I]	124.6 ± 56.2	112.0 ± 49.1
Glutamic pyruvic transaminase (GPT) [U/I]	16.6 ± 12.7	14.4 ± 7.7
Uric Acid [mg/dl]	5.5 ± 5	8.3 ± 3.8
Alkaline Phosphatase [U/I]	298.8 ± 156.3	233.4 ± 93.6
Gamma glutamyl transferase (γ-GT) [U/I]	22.3 ± 13.4	20.7 ± 18.7

All treatments not significantly different (P>0.05)

4.5.4 Maize DNA degradation (maize-DNA investigation in digesta and excreta samples)

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).

To investigate the passage and degradation of maize DNA in the different sections of the broiler GIT in both control and experimental groups, birds were slaughtered at different times after feed withdrawal. The maize specific fragments (using ZM1-F/ZM1-R/ZM1 primer-probe-system) were detected in all collected digesta samples from control and experimental chickens.

Cycle thresholds of maize specific fragments (ZM1-F/ZM1-R/ZM1 primer-probe-system) in control and experimental group digesta using real time PCR are demonstrated in Table 15. Figure 17 and 18 show the maize specific fragments obtained by using real time PCR in the digesta of control and experimental group as well.

Table 15. Cycle thresholds of maize specific fragments in control and experimental group digesta after feed withdrawal [C_T]

GIT-section	Control group			Experimental group					
	Feed withdrawal			Feed withdrawal					
	0h	4h	24h	0h	2h	4h	8h	12h	24h
Crop	26.8	0	0	22.9	26	0	0	0	0
Proventriculus	23.4	32.2	0	26.8	33.2	38.2	0	0	0
Gizzard	25.7	25	38.5	29	31.9	34.1	33.8	39.2	0
Duodenum	30.5	35.1	42.2	31.5	36.2	39.2	0	42.5	39.4
Jejunum	26.8	27.1	39.2	37.1	34.7	34.8	38	37.3	37.9
Ileum	31.9	33.4	0	36	31.2	29.8	42.1	35.8	0
Caeca and Rectum	36.4	40.5	36.9	41.6	36.3	39	34.4	0	37.9

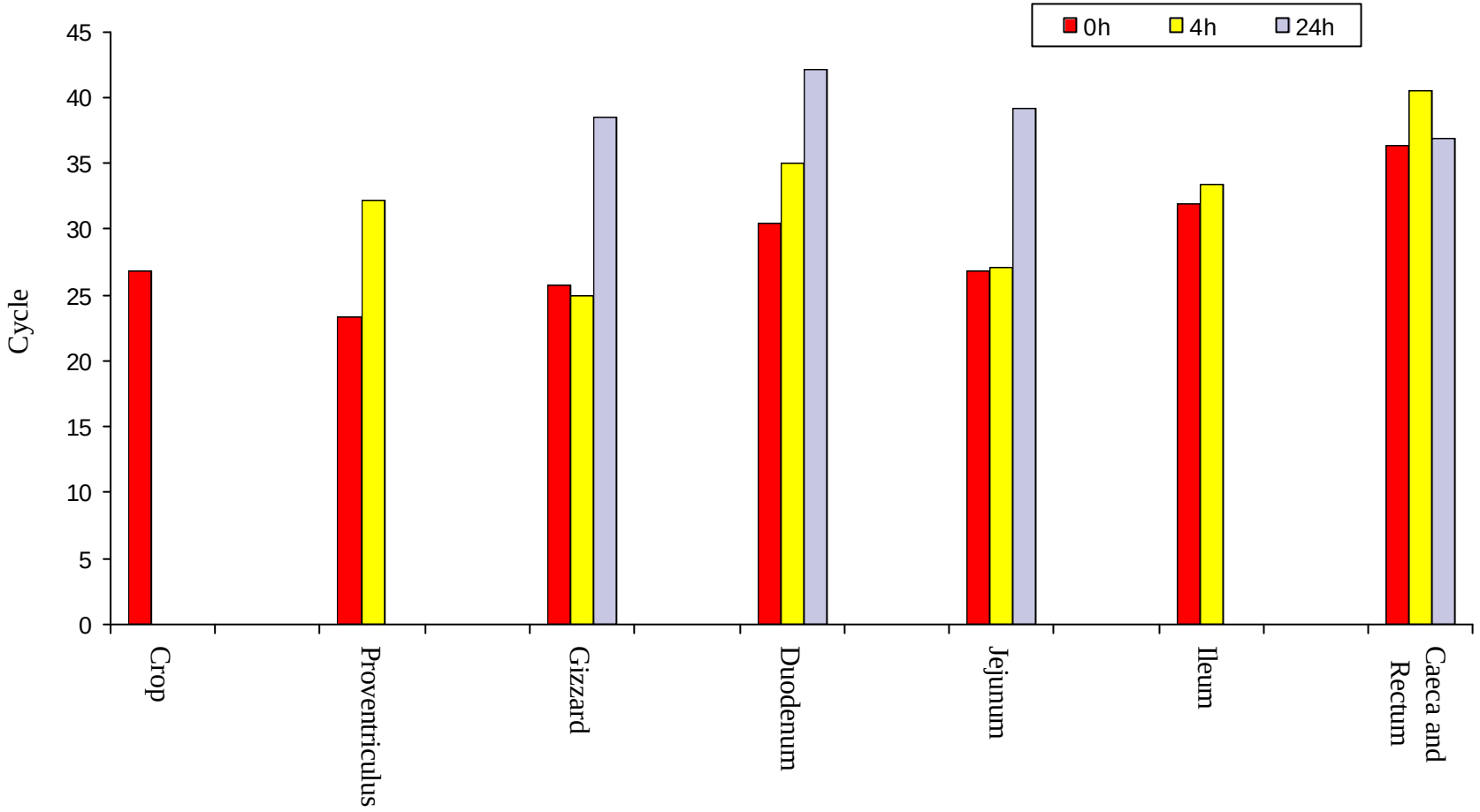

Figure 17. Maize specific fragments in control group digesta using real time PCR (cycle threshold)

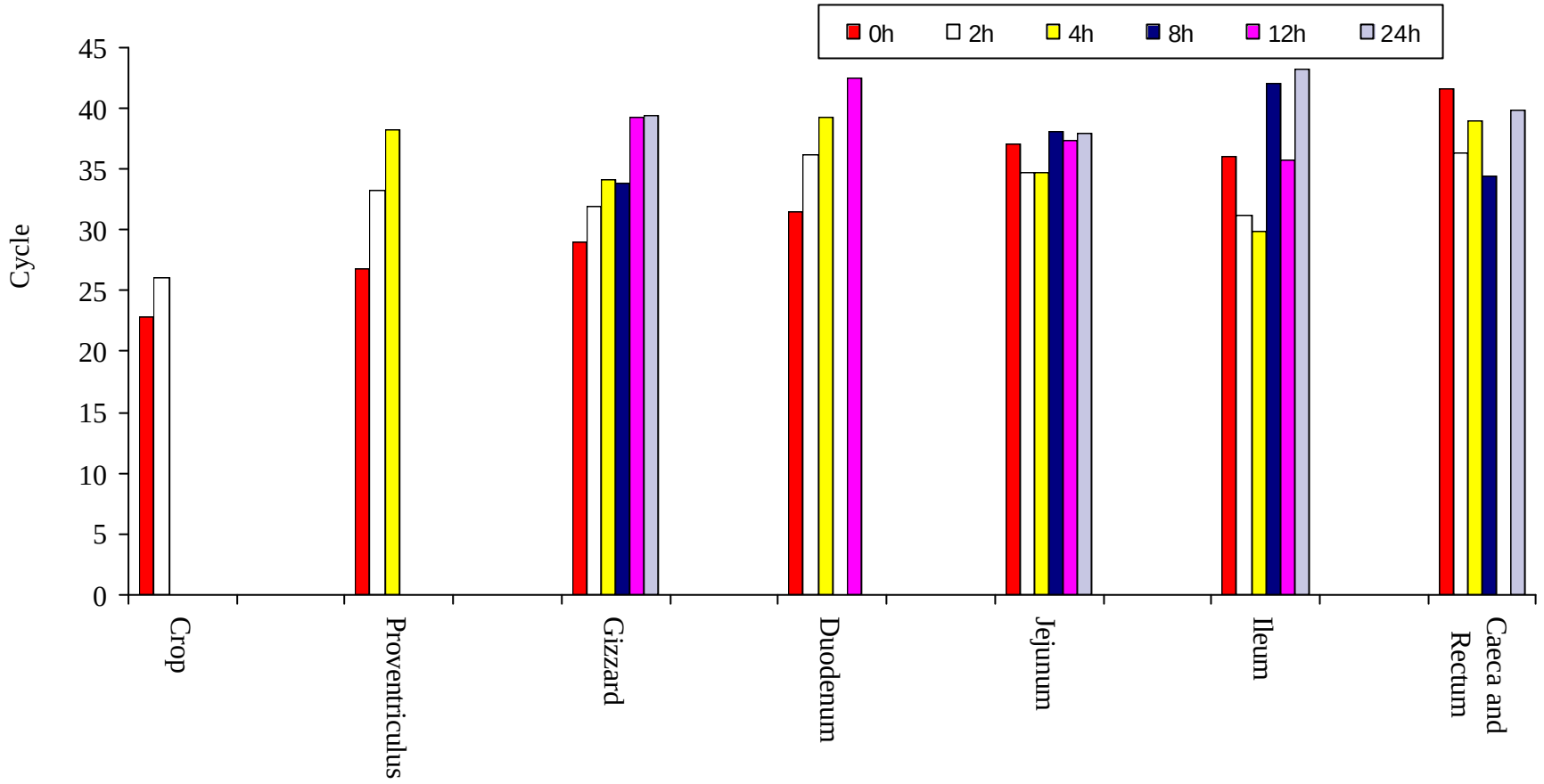

Figure 18. Maize specific fragments in experimental group digesta using real time PCR (cycle threshold)

Table 16 shows the relative Bt176 DNA concentration percent in digesta samples of the experimental group after feed withdrawal. In the digesta samples collected from the experimental group, Bt-maize DNA was also amplified using Cry2-F/Cry2-R/BTSYN primer-probe-system (Figure 19). 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 could also be detected in all faecal samples, which collected at 20 – 25 days of age, all tested samples gave positive results by using TaqMan PCR technology (Results not shown).

The Bt-maize DNA detected in the samples collected at 24h after feeding (Figure 19) demonstrated that the genetic construct in the Bt-maize resist digestion in broiler gut and subsequently seems to be not absorbed.

Table 16. Relative Bt176 DNA concentration in digesta samples of the experimental group after feed withdrawal [%]

GIT-section	**Feed withdrawal**					
	0h	**2h**	**4h**	**8h**	**12h**	**24h**
Crop[1]	100	80	0	0	0	0
Proventriculus	80	60	40	0	0	0
Gizzard	80	60	60	60	40	40
Duodenum	60	40	40	0	40	0
Jejunum	40	60	60	40	40	40
Ileum	40	60	80	20	40	20
Caeca and Rectum	20	40	40	60	0	40

1) Relative concentration to the content of the crop at 0h after feed withdrawal

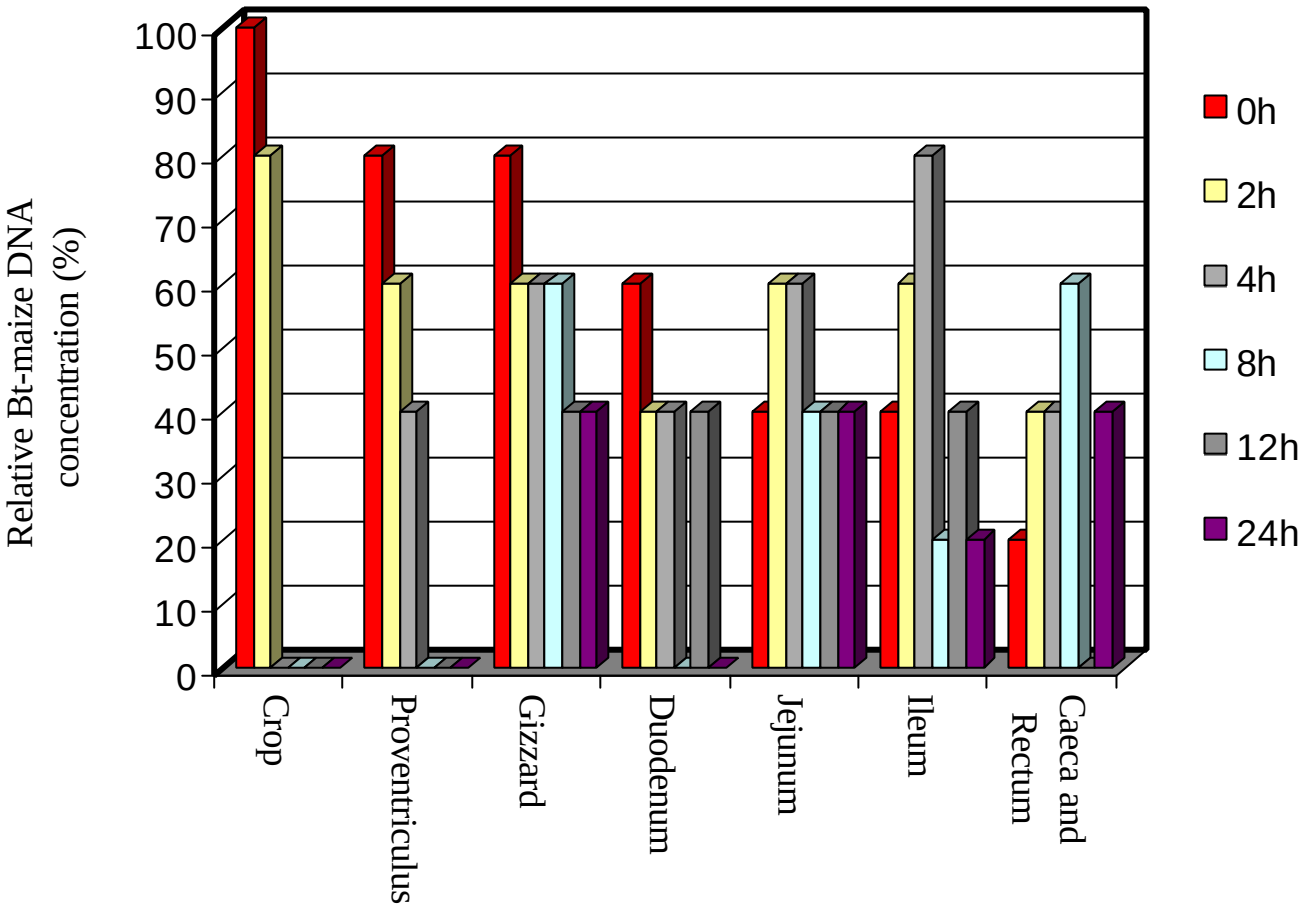

Figure 19. Degradation of Bt176 maize DNA in broiler GIT

4.5.5 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 (Figure 20). This primer-probe-system amplifies mammals and poultry chromosomally encoded *myostatin* gene (Laube et al., 2003). The obtained results confirmed that the DNA was successfully isolated from blood and different tissues as well as the isolated DNA able to be amplified during the PCR.

The Cycle threshold (C_T) generated by real-time PCR using MY-F/MY-R/MY-probe (primer-probe-system) is inversely proportional to the number of template copies (poultry chromosomally encoded *myostatin* DNA) 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 as described by Heid et al. (1996).

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.

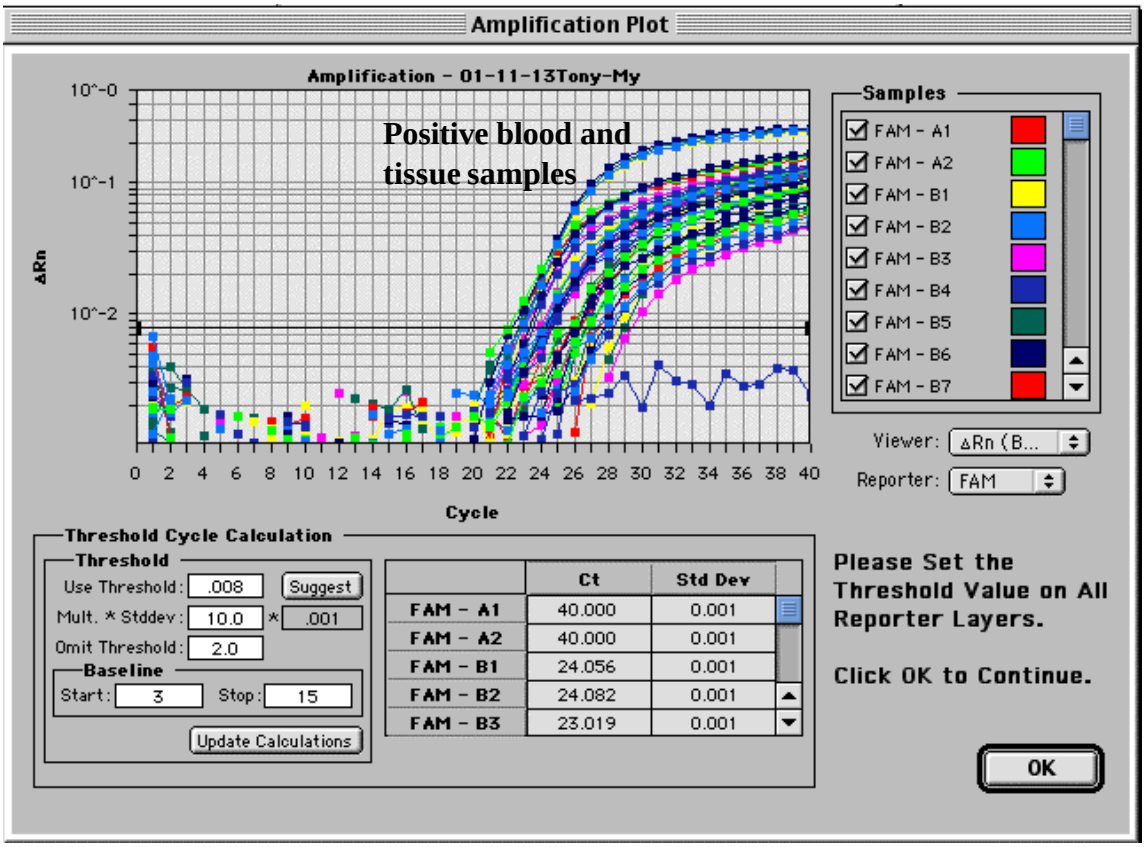

Figure 20. Amplification plots using MY-F/MY-R/MY-probe in real time PCR, products of poultry chromosomally encoded *myostatin* gene

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 (Figures 21 and 22). Interestingly, no more plant DNA amplification showed in the blood and tissue samples collected 24h after the last feeding in both groups (No amplification was detected).

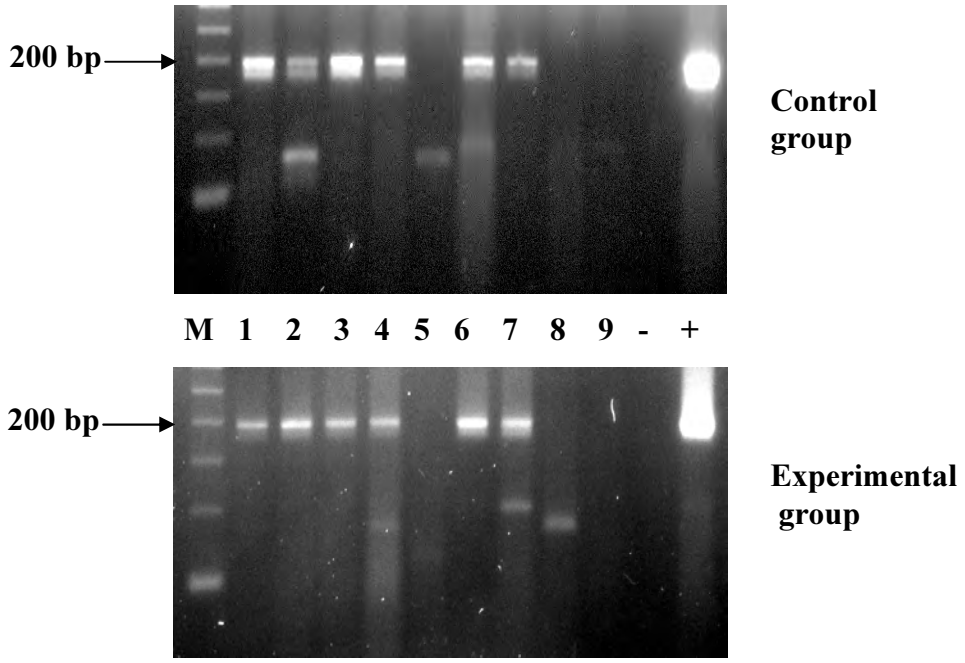

Figure 21. Two PCR assays detecting short maize chloroplast fragments (199 bp) in broiler blood, muscles and organs (directly after feed withdrawal "0h fasting")

M: 50 bp DNA-marker, 1: blood, 2: pectoral muscle, 3: thigh muscle, 4: liver, 5: heart muscle, 6: spleen, 7: kidney, 8: bursa, 9: thymus, -: negative control without DNA, +: positive control using standard maize DNA

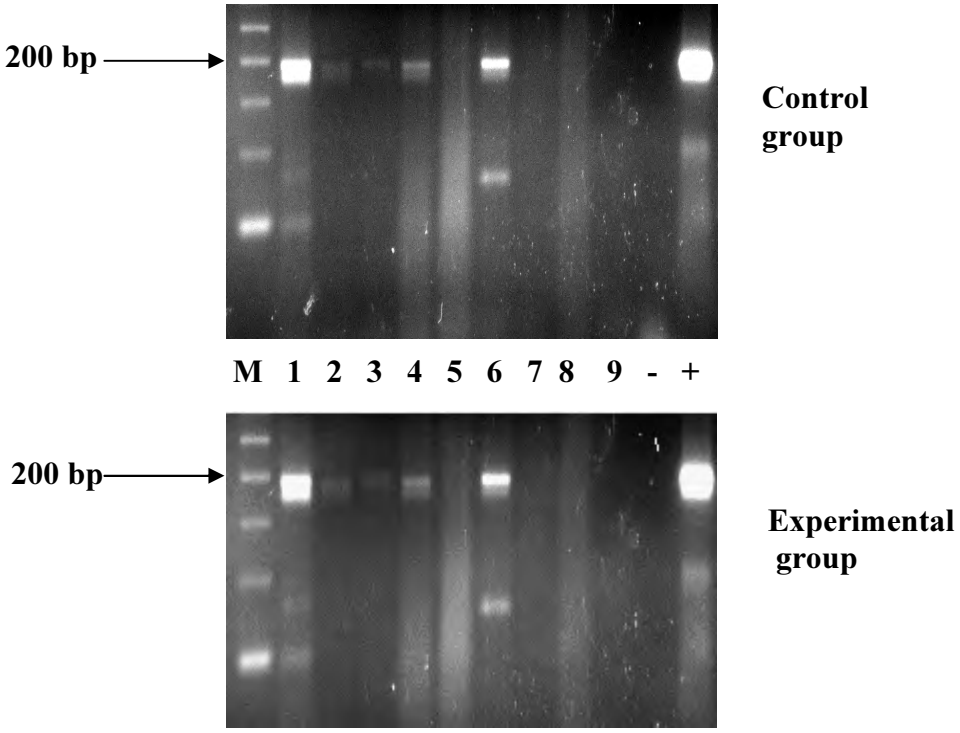

Figure 22. Two PCR assays detecting short maize chloroplast fragments (199 bp) in broiler blood, muscles and organs (4h after feed withdrawal)

M: 50 bp DNA-marker, 1: blood, 2: pectoral muscle, 3: thigh muscle, 4: liver, 5: heart muscle, 6: spleen, 7: kidney, 8: bursa, 9: thymus. -: negative control without DNA, +: positive control using maize standard DNA