Investigation on genetically modified soybean (RoundUp Ready) in goat nutrition: DNA detection in suckling kids

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ABSTRACT: The presence of plant DNA fragments in blood, kidney, hearth, liver, spleen and muscle tissue from suckling kids was investigated by using PCR approach. Fragments of high copy number chloroplast and low copy soybean lectin genes were found in several samples of kids whose mother were fed diet containing conventional (control) or transgenic soybean (treated). Only in treated group, fragments of 35S and CP4 *epsps* soybean genes were found in several samples.

Key words: Genetically modified soybean meal s.e., Kids, DNA transfer.

INTRODUCTION - Small fragments of plant chloroplast and species specific sequence were found in animal samples (Aeschbacher *et al.*, 2005; Tudisco *et al.*, 2006) confirming that plant DNA is not completely degraded by digestive processes. However, only Aeschbacher *et al.* (2002) and Chowdhury *et al.* (2003a) detected genetically modified (GM) fragments respectively in the liver, spleen and muscle of poultry and in the gastric and intestinal contents of pigs. In our previous research (Laudadio *et al.*, 2006) we found the chloroplast sequence in blood and tissues of lambs allowed to nurse the dam until weaning. However, no single copy feed fragment was detectable in lambs samples nor in the mothers milk and blood. Contemporaneously, we carried out a study on two groups of goats fed diet containing conventional or GM soybean. In blood and milk of both groups we found chloroplast (90% of subjects) and soybean *lectin* fragments (60%) but only in blood and milk of goats fed GM soybean we found 35S promoter (45% of subjects) and *epsps* fragments (30%). Of this last research, we discuss for brevity only the results of detection of the above fragments in tissues and blood from the kids allowed to nurse the dam until weaning.

MATERIAL AND METHODS - Twenty Cilentana goats were equally assigned to group C (control) and T (treated). The animals were fed diet constituted by hay and concentrate (16.5% CP and 0.90 UFL/kg, as fed), the latter containing soybean meal s.e. which was from conventional or genetically modified (RoundUp Ready®, tolerant to the glyphosate herbicide) beans for group C and T, respectively. After delivery, six kids were randomly collected from each group and allowed to nurse the dam, without any possibility to intake mothers diet. Immediately before the slaughtering (age: 60 ± 5 days; live weight: 11.2 ± 0.8 kg), blood (10 ml) and, subsequently, muscle tissue (left leg), liver, kidney, heart and spleen samples were collected from each kid. All the samples were divided in 3 aliquots and stored at -20°C. The sample preparation was carried out to avoid potential contaminations as described by Tudisco *et al.* (2006). As controls, DNA from conventional and GM soybean meal s.e. was extracted according to the Wizard extraction method (Promega). Tissue (25 mg) and blood (5 ml) samples were, respectively, extracted by using the "Nucleo-Spin Tissue" (Macherey-Nagel) according to users' manual and phenol/chloroform method. DNA concentration and quality were determined by spectrophotometer. Each aliquot was extracted in duplicate and stored at -20 °C until use. The quality of DNAs extracted were checked in a PCR reaction (Applied Biosystems) in order to amplify a conserved portion of caprine mtDNA (Bottero *et al.*, 2003). Subsequently, samples were monitored for the presence of the chloroplast sequence by using the Clor 1/2 primers designed on chloroplast *trnL* gene

sequence (Terzi *et al.,* 2004). Finally, in the samples from both groups of kids resulted positive for chloroplast gene, species specific primers for conventional and GM soybean were used: Le1n02 5/3 which amplifies the soybean *lectin* gene (Kuribara *et al.*, 2002), 35S 1/2 which amplifies the 35S CMV promoter (Lipp *et al.,* 1999) and CP4EPSPS 1/2 which amplifies a part of the specific gene sequence (*epsps*) that provide herbicide tolerance (Hernández *et al.*, 2003). Sequence, amplicon size and annealing temperature of all the primer pairs are shown in table 1. The PCR was done 3 times for each replicate and samples with positive results at least twice were judged as positive (Chowdhury *et al.*, 2003b)

Table 1. Sequence, amplicon size (bp) and annealing temperature (°C) of primer pairs used.

Primers	Sequence (5′-3′)	bp	°C
Cap 144	CGC CCT CCA AAT CAA TAA G	326	55
Cap 469	AGT GTA TCA GCT GCA GTA GGG TT		
Clor1	TTCCAGGGTTTCTCTGAATTTG	100	60
Clor2	TATGGCGAAATCGGTAGACG		
Le1n02-5	GCCCTCTACTCCACCCCCA	118	59
Le1n02-3	GCCCATCTGCAAGCCTTTTT		
35S 1	GCTCCTACAAATGCCATCA	195	56
35S 2	GATAGTGGGATTGTGCGTCA		
CP4EPSPS 1	GCA AAT CCT CTG GCC TTT CC	145	60
CP4EPSPS 2	CTT GCC CGT ATT GAT GAC GTC		

RESULTS AND CONCLUSIONS - Chloroplast fragments (figure 1a) were found in all samples of both groups in the following percentage: 91.6% (liver), 83.3% (kidney), 75.0% (muscle), 66.6% (spleen), 58.0% (heart), 50.0% (blood). In the samples positive for chloroplast, the fragments of *lectin* gene (figure 1b) was detected in: 36.4% (liver), 50.0% (kidney), 44.4% (muscle), 25.0% (spleen), 28.6% (heart), 16.6% (blood). Higher incidence of feed DNA fragments in tissues than in blood was found also by Tudisco *et al.* (2006a; 2006b). In addition, Phipps *et al.* (2003) in dairy cows found chloroplast fragments in milk but rarely in blood. Transgenic fragments were not found in all the samples of group C, while in group T (figure 2), fragments of 35S promoter and CP4 *epsps* gene were found respectively in liver (50.0% and 16.6%), kidney (80.0% and 40.0%), muscle (40.0% and 60.0%), spleen (25.0% and 25.0%), heart (25.0% and 75.0%) and blood (75.0% and 25.0%). Different findings between promoter and herbicide tolerance gene was found also by Alexander *et al.* (2006). The presence of feed plant DNA in suckling animals samples could suggest the hypothesis of transfer of gene fragments through the milk or during the gestation.

Figure 1. Representative electrophoretic analysis of chloroplast (a) and lectin fragments (b) in liver, kidney, heart, muscle, spleen and blood from group C (lanes 1 to 6, respectively) and T (lanes 7 to 12, respectively). In each panel, lane M contains a 100-bp DNA ladder, - is a negative control (non DNA template), and + is a positive control (soybean meal).

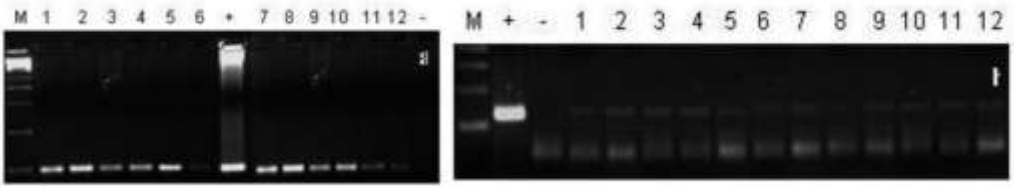
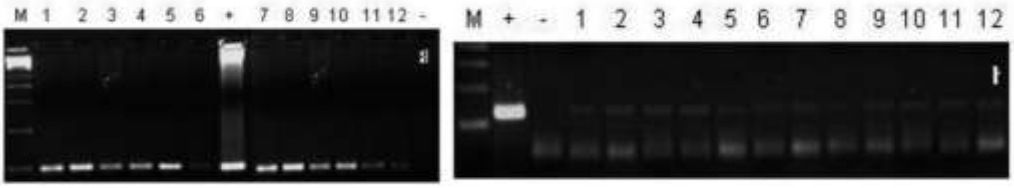


Figure 2. Representative electrophoretic analysis of 35S promoter (a) and CP4 epsps (b) fragments in liver, kidney, heart, muscle, spleen and blood from group T. In each panel, lane M contains a 100-bp DNA ladder, - is a negative control (non DNA template), and + is a positive control (RR soybean meal).



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