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Genetically modified soybean in a goat diet: Influence on kid performance☆

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ABSTRACT

The in vivo and *post mortem* performance and serum immunoglobulin G (IgG) concentration in kids born from goats fed conventional (group C) or genetically modified (group T) soybean meal were evaluated. The goat colostrum quality, in terms of chemical composition, as well as immunoglobulin concentration, and the presence of feed DNA fragments were also investigated. Kid birth weights were similar, while significantly ($P < 0.05$) higher in those born from goats in group C at day 30 and at slaughtering. In addition, kids from mothers fed conventional soybean recorded significant ($P < 0.05$) higher height at the withers and chest width. Concerning the post mortem measurements, only carcass weights were significantly affected by the treatment resulting in lighter T kids ($P < 0.05$). Colostrum from the treated groups recorded a significantly ($P < 0.01$) lower percentage of protein and fat. Similarly, both chemical parameters significantly differed in milk collected 15 days after kidding, although these differences disappeared in the successive samplings. Both colostrum and kids serum IgG concentration were significantly ($P < 0.01$) lower in the treated groups. Transgenic target DNA sequences (35S and CP4 EPSPS) were not detected in colostrum from goats that received a diet containing conventional soybean meal. By contrast, transgenic DNA fragments were amplified ($P < 0.05$) in samples from goats that received the transgenic soybean.

1. Introduction

Several studies on the influence of feeding genetically modified (GM) plants on the performance of various animal species have been performed. Results have also been summarized and discussed in review articles (Aumaitre et al., 2002; Flachowsky et al., 2007, 2012). Concerning ruminants, the inclusion in the diet of ingredients derived from a wide range of GM plants did not affect feed intake, milk yield and composition. Moreover, comparable performances in growing animals fed GM or conventional plants have been reported (EFSA, 2008).

It is well known that colostrum plays a critical role in ruminants, since its early ingestion is the one method to produce maternal antibodies (Lombardi et al., 1996). An adequate passive transfer of immunity, determined by measuring serum immunoglobulin G (IgG) concentration, is a critical determinant of the short-term health and survival of neonatal ruminants. It has been reported that, for instance, to ensure adequate passive transfer of immunity, kids should receive a sufficient volume and concentration of colostrum within the first 12 (Massimini et al., 2007) to 24 h of life (Arguello et al., 2004; Massimini et al., 2006; Mastellone et al., 2011).

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An inadequate ingestion or absorption of colostrum IgG leads to a secondary immunodeficiency condition, termed failure of passive transfer (FPT), that predisposes ruminant neonates to the development of bacterial septicaemia and common neonatal diseases (Mastellone et al., 2011). Calves with FPT have an increased risk of death until at least 10 weeks of age and, in neonatal calves <7 days old, this risk has been associated with a serum IgG concentration of <10 mg/mL (Tyler et al., 1998; Weaver et al., 2000; Barrington and Parish, 2002).

Passive transfer of immunity seems also to have a predictive value for health and productivity outcomes (i.e., subsequent growth and production) in juvenile calves, lambs, and kids both before and after weaning (Massimini et al., 2007). In dairy calves, serum IgG concentration 24–48 h after calving was a significant source of variation in average daily gain (ADG) through the first 180 days (Robison et al., 1988). Additionally, it was showed that passive transfer status was a significant source of variation in growth performance in buffalo calves. Therefore, maximizing passive transfer of immunity by allowing calves to nurse the dam can increase growth performance during the first month of life (Mastellone et al., 2011).

All these data underline the importance of passive transfer of IgG in ruminants. Therefore, the possible influence of feeding in determining the composition and quality of colostrum may be critical for the achievement of adequate passive immunity and, as a consequence, for the best growing performances. The possible effects of GM feeding in changing some protein activity and synthesis, mainly at cellular level, have been recently underlined by several researchers (Tudisco et al., 2006, 2010; Trabalza-Marinucci et al., 2008; Mastellone et al., 2011, 2013).

To current knowledge, no data are available concerning the influence of GM feeding on colostrum composition in ruminants. The aims of this study were thus to evaluate:

1. colostrum quality, in terms of chemical composition and IgG concentration, in an autochthonous goat population called 'Cilentana' bred in Cilento (Salerno province, Southern Italy), fed GM soybean;
2. in vivo and *post mortem* performance and serum IgG concentration in kids fed only dams milk;
3. presence of feed DNA fragments in the colostrum.

2. Materials and methods

2.1. Diets, animals and feeding

The trial was performed on a farm located at Casaletto Spartano, Salerno province, Southern Italy, at 832 m above sea level where 300 goats of an autochthonous goat population, called 'Cilentana', are bred. Two months after kidding goats generally have free access to pastures (9.00 am to 4.00 pm), constituted by 60% Leguminosae (*Trifolium alexandrinum*, *Vicia* spp.) and 40% Graminee (*Bromus catharticus*, *Festuca arundinacea*, *Lolium perenne*).

The present experiment was performed on 40 male kids born from four groups of pluriparae goats, homogeneous in parity and milk production during the previous lactation. Sixty days before kidding, goats of each group ($n = 10$) were housed in separate sheds and fed isoenergy and isoprotein diets consisting of oat hay and commercial concentrate. The latter containing solvent extracted (s.e.) soybean meal (13% or 20% of concentrate DM) which was from conventional or GM (MON40-3-2) soybean for groups C (control; C13 and C20) and T (treated; T13 and T20), respectively.

Table 1
Chemical composition (g/kg DM) and nutritive value (UFL/kg DM) of hays and concentrates.

	Oat hay	Alfalfa hay	C13 and T13[a]	C20 and T20[b]
Crude protein	55.0	150.0	180	180
Ether extract	19.0	20.0	30.0	27.0
NDF	682.0	493.0	270.0	280.3
ADF	451.0	341.0	115.0	118.0
Lignin	61.0	50.0	30.0	33.0
UFL	0.47	0.72	1.03	1.00

[a] Soft wheat bran 30; soybean solvent extracted (conventional or RR®) 13; corn meal 13; sunflower meal 10.5; citrus pulp 8; sugar beet pulp 7.9; corn gluten feed 7; sugarcane molasses 7.5; $CaCO_3$ 1.5; $CaHPO_4$ 0.7; vitamins 0.2; NaCl 0.7.

[b] Soft wheat bran 31; soybean solvent extracted (conventional or RR®) 20; corn meal 14; citrus pulp 8; sugar beet pulp 8.9; corn gluten feed 7; sugarcane molasses 8; $CaCO_3$ 1.5; $CaHPO_4$ 0.7; vitamins 0.2; NaCl 0.7.

MON40-3-2 is tolerant to the glyphosate family of herbicides by expressing transgenic DNA from the CP4 strain of *Agrobacterium tumefaciens* (CP4 EPSPS), encoding 5-enolpyruvilshikimate-3-phosphate synthase protein (glyphosate-tolerant soybean GTS 40-3-2) (Padgette et al., 1995). A polymerase chain reaction (PCR)-end point reaction confirmed the presence of p35S and CP4 EPSPS specific transgenes (MON40-3-2) in the treated diet, as well as their absence in the control diet. Goats received oat hay ad libitum, while the concentrate was administered in amounts of 200, 300 and 400 g/head per day, 60, 30 and 15 days before kidding, respectively. After kidding, administration of the concentrate was gradually increased up to 700 g/head per day and oat hay was replaced by alfalfa hay, in order to increase the diet protein content. Animals had free access to the water. The chemical composition (Van Soest et al., 1991; AOAC, 2000) and net energy calculated according to INRA (1978) of hays and concentrates as well as ingredients of concentrate are set up in Table 1.

The trial was performed according to Animal Welfare and Good Clinical Practice (Directive 2010/63/EU) and was approved by the local Bioethics Committee. Ten male kids were randomly selected from twin deliveries in each group. Immediately after kidding kids were separated from the mothers and put into individual cages in a separate room. Each kid was fed only colostrum/milk from its mother, by using a milk feeder. Colostrum/milk was collected from each goat into sterile tubes with aseptic techniques and administered twice/day (50 mL/kg of body weight, BW), until 24 h before slaughtering.

2.2. Sampling collection and pre-treatment

Within 1 h from kidding, individual samples of colostrum were collected and divided in aliquots: (1) for IgG concentration, colostrum (10 mL) was first centrifuged at 4000 × *g* for 15 min to remove fat and sediments and then centrifuged at 20,000 × *g* for 30 min. The intermediate layer was withdrawn for analysis. (2) For DNA analysis, 10 mL colostrum was incubated at 4 °C overnight and centrifuged (2000 × *g* for 20 min at 4 °C) to separate the cream, skimmed colostrum and sediment; only the sediment fraction was subjected to DNA extraction. (3) No pre-treatment was performed for chemical composition (30 mL).

Twenty-four hours after kidding, blood was collected from the jugular vein of the kids. Samples were centrifuged at 2500 × *g* for 10 min and the serum collected for IgG concentration.

One hundred mL of milk (obtained by mixing the production of the two daily milkings) from each goat were fortnightly collected and analyzed for protein and fat content by the infrared method using a Milko Scan 133B (Foss Matic, Hillerod, Denmark), standardized for goat milk. Milk samples were collected at days 15, 30, 45, 60 after kidding.

2.3. IgG assay

IgG concentration was determined by use of a commercially available RID assay for goat IgG, according to the manufacturer's specifications. Briefly, 5 μL of serum or colostrum were added to 1 well of a 48-well plate containing anti-goat IgG antiserum, Tris-buffered saline solution, and 0.1% sodium azide in a 1.5% agarose matrix. Three reference standards (2.5, 10, and 20 mg of goat IgG/mL) included in the kit were tested concurrently with each sample. The plate was incubated at room temperature (approx