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Plasma immunoglobulins levels in dry and lactating goats fed diets containing tomato and cucumber waste fruits

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Abstract. In the last years there has been an increasing interest in the use of horticulture wastes in ruminants feeding. Fruits and vegetables are good sources of biologically active phytochemicals which could modulate host immunity. Two experiments were conducted to analyse the effects of diets including wastes of tomato and cucumber fruits on plasma IgA and IgG levels in goats. Four experimental diets were formulated and tested in a Latin square experimental design. The control diet (CON) consisted of alfalfa hay plus concentrate in a 1:1 ratio and the other three diets were formulated for dry and lactating goats by replacing 50 or 35%, respectively, of the concentrate with feed blocks including wastes of tomato fruits (TOM), cucumber (CUC) or barley (BAR). In Experiment 1, four dry non-pregnant rumen-cannulated goats (32.1 ± 5.52 kg BW) were used, and in Experiment 2 experimental animals were eight lactating goats (39.4 ± 5.39 kg BW). Blood samples were taken from each animal after 39 days of adaptation to the corresponding diet. Plasma samples from both experiments were analyzed for IgA and IgG using commercial ELISA kits. In Experiment 1, there were no differences between diets in plasma immunoglobulin levels ($P>0.73$ and 0.95 for IgA and IgG, respectively). No effect of diet was either observed in Experiment 2 ($P=0.47$ and 0.45 for IgA and IgG, respectively). The results indicate that including wastes of tomato and cucumber fruits in the diets of goats did not affect animals' plasma immunoglobulin levels.

Keywords. Goat – IgA – IgG – Horticulture wastes.

Niveaux plasmatiques d'immunoglobulines dans de chèvres sèches et en lactation alimentées avec des déchets de tomates et de concombres

Résumé. *Dans les dernières années il y a eu un intérêt croissant pour l'utilisation des déchets de l'horticulture dans l'alimentation des ruminants. Fruits et plantes sont de bonnes sources de composés phytochimiques biologiquement actives qui pourraient moduler l'immunité de l'hôte. Deux expériences ont été menées pour analyser les effets de l'alimentation incluant des déchets de fruits de tomates et de concombres dans l'alimentation des chèvres sur leurs niveaux plasmatiques d'IgA et IgG. Quatre régimes expérimentaux ont été formulés et testés suivant un design expérimental en carré latin. Le régime contrôle (CON) était composée de foin de luzerne, plus concentré dans un rapport 1 : 1 et les trois autres régimes étudiés dans des chèvres secs et allaitantes en remplaçant 50 ou 35%, respectivement, du concentré avec des blocs d'alimentation qui incluaient de fruits de tomate (TOM), concombre (CUC) ou de l'orge (BAR), respectivement). Dans l'expérience 1, quatre chèvres sèches et canulées dans le rumen (32,1 ± 5,52 kg BW) ont été nourris avec les quatre diètes études. Dans l'expérience 2, les animaux expérimentaux étaient huit chèvres en lactation (39,4 ± 5,39 kg BW). Des échantillons de sang ont été prélevés sur chaque animal après 39 jours d'adaptation au régime correspondant. Les échantillons de plasma des deux expériences ont été analysés pour IgA et IgG en utilisant des kits ELISA commerciaux. Dans l'expérience 1, il n'y avait pas de différences entre les régimes dans le niveau d'immunoglobulines (P>0,73 et 0,95 pour les IgA et les IgG, respectivement). No effet du régime a également été observée dans l'expérience 2 (P = 0,47 et 0,45 pour les IgA et IgG, respectivement). Les résultats indiquent que les régimes avec des blocs incluant les déchets de tomates et de concombres n'a pas affecté les niveaux d'immunoglobuline de plasma des animaux.*

Mots-clés. *Chèvre – IgA – IgG – Déchets de l'horticulture.*

I – Introduction

Consumers of feeds from animals concern more and more on both preventing and controlling animal diseases without chemical drugs (Krehbiel *et al.*, 2003) and decreasing the environmental impact of animal production by using natural components of diets. Secondary plant compounds such as saponins, tannins or essential oils may be potential feed additives to maintain healthy animals and improve ruminant production (Patra and Saxena, 2009). Additionally secondary plant compounds may reduce methane emissions diminishing the environmental impact of animal production, especially that based on ruminants. Therefore, for the future, feeding with feedstuffs with bioactive compounds could be one way of improving livestock production and health together with the product quality (Nielsen and Thamsborg, 2005).

Intensive agriculture in greenhouses is increasing especially in the Mediterranean countries, accounting for 15% of the total world production and a great part of the produced vegetables being supplied to the European Northern countries. In some areas of the Mediterranean countries animal production is limited by the scarcity and poor quality of natural pastures, thus creating the need of finding unconventional local and low cost raw materials for animal feeding different from natural pastures or conventional feeds that must be imported (Molina-Alcaide *et al.*, 2010). Greenhouse horticulture in Spain generates huge amounts of by-products, which are mainly discarded fruits of tomato (about 350,000 t/year) and cucumber fruits (about 60,500 t/year). The hypothesis driving the present study was that diets including wastes from greenhouse horticulture may have a modulatory effect on goats' immune system without affecting or even improving ruminal fermentation and animal productivity. Therefore in the present work the effect of different diets including greenhouse horticulture wastes (fruits of tomato and cucumber) on the levels of immunoglobulins A and G in plasma of dry and lactating goats was studied.

II – Material and methods

Tomato and cucumber waste fruits were collected at the Plant of Wastes Treatment in Motril (Granada, Spain), which gathers all the wastes from greenhouse horticulture in the Granada coast. Fruits were cut and kept at -20°C before being used for feed blocks preparation as described by Romero-Huelva and Molina-Alcaide (2013). Feed blocks including waste fruits of tomato, cucumber or barley grains were prepared and their ingredients composition is shown in Table 1.

Table 1. Ingredients composition (g/kg dry matter) of feed blocks including tomato fruits, cucumber and barley grains

	Tomato	Cucumber	Barley grains
Barley	–	–	83
Tomato	129	–	–
Cucumber	–	69	–
Wheat straw	598	639	631
Quicklime	81	88	85
NaCl	49	52	51
Sunflower meal	97	104	102
Urea	35	36	37
Vitamin-mineral mixture	11	12	11

Four experimental diets were formulated. The control diet (CON) consisted of 1:1 alfalfa hay:concentrate, and the other three diets were formulated for dry and lactating goats by replacing 50 or 35% of the concentrate with feed blocks including wastes of tomato fruits (TOM), cucumber (CUC) or barley grains (BAR), respectively. The chemical composition of the diets is shown in Table 2.

Table 2. Chemical composition of experimental diets (g/kg dry matter)†

	CON	TOM	CUC	BAR
DM, g/kg fresh matter	907	920	925	925
OM	898	859	855	860
Ether extract	20.6	12.3	11.9	12
NDF	462	502	494	507
ADF	259	311	308	315
ADL	56.7	65.1	65.1	66.6
Crude Protein	151	155	157	156
NSC††	264	190	192	185
GE, MJ/kg of DM	17.6	16.7	16.5	16.5

† CON: control diet; TOM: diet including tomato feed blocks; CUC: diet including cucumber feed blocks; BAR: diet including barley grain feed blocks.

†† NSC: non-structural carbohydrate calculated as OM - (Ether extract+ Crude protein + NFD).

2. Experiment 1

Four adult dry non-pregnant rumen-fistulated Murciano-Granadina goats were used in a 4 x 4 Latin square experimental design with four 39-d experimental periods. In each period one of the 4 experimental diets was fed to one of the animals. The CON diet was supplied to meet the energy requirements of this breed. No refusals for alfalfa hay and concentrate were observed for any of the dietary treatments. Feed blocks were supplied *ad libitum*. After 25 days of adaptation to the corresponding diet, blood samples were taken on day 39 by jugular venipuncture and collected in BD Vacutainer tubes with 170 UI of Lithium Heparin. After being mixed by inversion and centrifuged at 1800 g for 5 minutes at 4° C, the plasma was transferred using appropriate security measures to 2 mL Eppendorf tubes and stored at -80° C until analysis for IgA and IgG levels.

Aliquots of hay and concentrate supplied to the animals were collected and stored at -20°C for chemical analyses. Feed block refusals from each animal were collected daily in the morning, weighed and aliquots of refusals (30%) were stored at -20°C for analysis of chemical composition as described by Romero-Huelva and Molina-Alcaide (2013).

3. Experiment 2

Eight Murciano-Granadina goats in the middle (50 days in milk) of the third lactation were used. Four 39-d experimental periods were carried out in a 4 x 4 Latin square experimental design. In each period, 2 goats were randomly assigned to one of the four dietary treatments. Feed blocks were supplied *ad libitum* and refusals were taken daily and analysed as described in Experiment 1. After adaptation to the corresponding diet, blood samples were taken on day 39 as previously described and plasma was analysed for IgA and IgG levels.

4. Immunoassay and statistical analyses

Commercial kits (Bethyl Laboratories Montgomery, TX USA) were used for IgG and IgA quantification in plasma samples (Goat IgG Elisa Quantitation Set and Goat IgA ELISA Quantitation Set, respectively). Costar 3590 96-well EIA / RIA High Binding PS plates were used. The absorbance was read in a microplate reader (Microplate Reader model 550 from Biorad Laboratories, Veenendaal, The Netherlands) with a filter at 450 nm. According to the manufacturer protocol, the absorbance of the highest serum concentration should be between 1.8 and 2.2 OD. Samples noise was reduced by 5 washes using a LT-3000 micro-plate washer (Labtech International Ltd., Uckfield, UK), as suggested in the manufacturer protocol. The OD value for the blank was from 0.065 to 0.090 OD. The desired range of quantification was in the linear region of the curve, which was between 15.62 and 225 ng/mL as suggested in the protocol. Concentration values outside this range were repeated using either higher or lower dilutions. The absorbance values were recorded using the Biorad microplate Manager 4.0 software, and the samples giving a relative standard deviation greater than 10% were repeated. The same software was used to assess the concentration in undiluted goat serum, that was expressed as mg/mL for IgG and as ng/mL for IgA.

Data were analyzed as repeated measures ANOVA using the PROC MIXED of SAS (SAS Inst. Inc., Cary, NC). The effects of diet and period were considered fixed and animal was considered random. Differences were considered significant at P<0.05, and P<0.10 values were declared as trends and discussed.

III – Results and discussion

The concentration of total IgA and IgG (Table 3) in the plasma of goats were in the range of physiological levels for adult goats (Bach *et al.*, 2010). No effects of the inclusion of feed blocks containing waste fruits of tomato or cucumber on IgA and IgG plasma levels were observed either in dry (P = 0.906 and 0.983, respectively) or lactating goats (P = 0.544 and 0.622, respectively).

Table 3. Total IgA and IgG antibody levels in plasma of dry and lactating goats fed the experimental diets (n = 4 and n = 8, respectively)

	Diet†					
	CON	TOM	CUC	BAR	SEM	*P*-value
Dry goats						
IgA, ng/mL	15.5	15.9	15.4	16.8	0.371	0.906
IgG, mg/mL	8.87	9.27	8.95	8.79	0.191	0.983
Lactating goats						
IgA, ng/mL	68.3	74.9	67.0	75.1	3.29	0.544
IgG, mg/mL	24.4	26.0	27.1	26.0	0.782	0.622

† CON: control diet; TOM: diet including tomato feed blocks; CUC: diet including cucumber feed blocks; BAR: diet including barley grain feed blocks.

Some dietary strategies in ruminant´s feeding can result in changes in the ruminal microbiota, which could modify the host immunity (Shokrollahi *et al.*, 2013). One of the most important immune variables is the immunoglobulin concentration (mainly IgA and IgG), as immunoglobulins play an important role in host defense mechanisms against infections and their levels might rise sharply during the immune response (Hernández-Castellano *et al.*, 2015). Thus, the absence of changes in the immune response due to dietary treatments indicates that the inclusion of feed blocks containing waste fruits of tomato or cucumber did not promote rumen microbial stress with potential pathological consequences.

IV – Conclusions

Our study showed that the inclusion of greenhouse wastes in goat´s diet did not promote changes on the plasma IgA and IgG levels, thus suggesting that diets including tomato or cucumber feed blocks may not compromise the health status of the animals.

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