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Relevance of immune response against resident and not resident commensal strains for the definition of strategies of probiotic supply in the diet of weaning pigs

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RIASSUNTO – Importanza della risposta immunitaria nei confronti di commensali residenti e non, per la definizione di strategie d'impiego di probiotici nella dieta del suino in svezzamento. *Con l'impiego di probiotici spesso si osserva una maggiore risposta umorale, ma non è documentato se l'animale d'allevamento reagisca anche contro lo stesso probiotico. Sono stati analizzati campioni di sangue e saliva raccolti in due prove di somministrazione di probiotici a suini in svezzamento: A) Lactobacillus rhamnosus GG, non tipico del suino; B) Lactobacillus sobrius 001^T, isolato dal suino. Anticorpi probiotico specifici sono stati riscontrati nel siero dei soggetti prima del trattamento (prova A) e nei gruppi di controllo, nonostante l'assenza di DNA ceppo-specifico nel loro contenuto intestinale (prova A e B). Utilizzando un test ELISA su siero o saliva preincubati con uno o l'altro dei lattobacilli, si è visto che ciascuno di questi lega parte delle IgA specifiche per l'altro. Quindi batteri con differente affinità per il suino possono presentare attività immunologia crociata reciproca. Nella definizione dell'impiego di un probiotico nella dieta del suinetto si deve valutare con attenzione la presenza di anticorpi già attivi prima della sua somministrazione.*

Key words: weaning pig, probiotics, feeding, immunity.

INTRODUCTION – Copious research data from trials in vitro and on experimental animals are available showing that the gut immune system could be down- or up- regulated in order to improve the overall health of the host. In some cases it is claimed that humoral immunity was increased, with special emphasis on the stimulation of specific response against certain pathogens. However the development of specific immune response towards commensal bacteria and probiotics is less documented. Indeed a prolonged secretion of immune globulins is a cost for the host and could also reduce the chance of survival of the probiotic in the gut. Changing levels of *Lactobacillus*-species specific IgA were observed in mice mono-associated with 2 lactobacilli showing similar in vitro adhesion patterns (*L. johnsonii* NCC 533 or *L. paracasei* NCC 2461) (Ibnou-Zekri *et al.*, 2003). Nevertheless the variations of Lactobacillus-species specific IgA are in general not studied in normally fed producing animals. We wanted to assess at different times after weaning the IgA activity against a pig-specific and a pig-not specific strain. Secondly we wanted to verify if part of this IgA strain-specific activity was partially related to cross-reactivity between two different *Lactobacillus*-species.

MATERIALS AND METHODS – Data refer to blood and saliva samples collected at different times during two different trails in witch *L.rhamnosus* GG (Lb-R) (Trial A) and strain 001^T of the new species *Lactobacillus sobrius* (Lb-S) (Trial B), were respectively supplemented to pigs. The first microbe is a probiotic approved for human use and is considered to be not commensal of pig, while the second was isolated at the University of Wageningen in piglets fed fermentable diets. In both trials, pigs, weaned at 21 days of age, were fed control diet or control diet plus 10^{10} Colony Forming Units (CFU) probiotic/day. Pigs were orally challenged with 10^{10} CFU *E. coli* K88ac O148 (F4) on day 7, and sacrificed after another week. IgA specific activities were measured as reported by Bosi *et al.* (2004), except that microplate was coated with probiotic bacteria suspended in carbonate buffer at an optical density of 1.0 at 660 nm. The presence of both lactobacilli in gut samples was tested by PCR. Total DNA was extracted using QIAamp® DNA Stool Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's manual. 80 ng of total DNA extracted from each sample were amplified in a thermal cycler (PTC-100 Programmable Thermal Controller). For PCR conditions and strain-specific primers we referred: *L. rhamnosus* GG, to Brandta and Alatossava (2003); *L. sobrius*, to Konstantinov *et al.* (submitted to Appl. Environ. Microbiol.).

To test the immune cross-reactivity between different *Lactobacillus*-species we implemented the procedure of Shu *et al.* (1999). For trial A, two cell suspensions with Lb-R and with Lb-S were prepared suspending the lyophilized bacteria in PBS at an optical density of 1.0 at 660 nm. From each serum or saliva sample, from pigs fed Lb-R diet, 2 sub samples were obtained and added respectively with Lb-R and Lb-S suspensions. After mixing, both the suspensions were incubated for 1h at 37°C in a shaking water bath and then centrifuged at 4°C for 30 min (11000 x g). The supernatants were filtered through a 0.4 μm membrane and tested by ELISA for the residual antibody activity against Lb-R. Therefore in different wells of the microtitre plate were added: - serum absorbed with Lb-S (Test); - serum absorbed with Lb-R (Negative Control); - unabsorbed serum (Positive Control). The cross reactivity index (CRI,%) was calculated as: (Positive Control-Test)/(Positive Control-Negative Control) x 100. On a scale of 0-100%, a CRI of 100% would be complete cross-reactivity and 0% would be nil identity between Lb-R and Lb-S. For the trial B, the position of Lb-R and Lb-S were inverted.

RESULTS AND CONCLUSIONS – Figure 1 shows that seric IgA anti-*L. rhamnosus* GG were present at all the samplings, even before the dietary supplementation with *L. rhamnosus* GG and in the control group. This contrast with the fact that *L. rhamnosus* GG is not considered a strain typical of the pig. Indeed, cecum samples obtained from control subjects were negative for the presence of DNA from *L. rhamnosus* GG (at the contrary, most of the probiotic-fed pigs were positive). A similar observation was done in the second trial, where seric IgA anti L. sobrius strain 001^T were also detected in control pigs, that were negative in ileum for the presence of this particular strain (not in figure). All these observations can be explained by the presence of a cross reactivity between different bacteria. If the values of probiotic specific IgA (Figure 1) are expressed on total IgA content and as a different between difference times of samplings, we can see that the strain-specific IgA relative content in the *Lactobacillus* group increased between start and 1st week; this was not observed after and for the control group. This observation shows that there was an additional short-term specific immune response in the probiotic group, in agreement with observations on germ-free piglets fed with non-pathogenic *E. coli* (Cukrowska *et al.*, 2001).

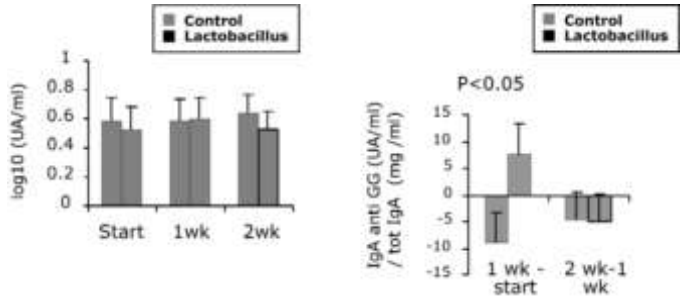

Figure 1. Effect of dietary supplementation with *L. rhamnosus* GG on seric IgA anti-*L. rhamnosus* GG and on time variation of relative content of IgA anti-*L. rhamnosus* GG on total seric IgA (LSM+SEM).

Table 1. Cross reactivity index (CRI) evaluated by pre-absorption test (Means+SD).

Trial	Samples	N	Antibodies against	Pre-absorbed with	CRI
A	Blood, at start	3	*L. rhamnosus* GG	*L. sobrius* strain 001^T	79.5 + 3.5
A	Blood, at sacrifice	3	*L. rhamnosus* GG	*L. sobrius* strain 001^T	92.1 + 7.8
B	Saliva, after 1 week of probiotic supply	4	*L. sobrius strain* 001^T	*L. rhamnosus* GG	67.1 + 14.5

Cross reactivity index (Table 1) evaluated by pre-absorption test for different samples (blood or saliva) and for different specific IgA's was high. There is a consistent debate in the literature about the presence of antibodies that apparently react with many different microbes. One possible explanation is the similarity of some amino acid and sugar motifs in the structure of the S-layer that protects Gram+ cells; a second hypothesis is that part of these IgA's are "polyreactive" or "natural". Then it is not yet clear the functional significance of the presence of IgA against commensals: it could constrain these bacteria inside the gut lumen, but it could also improve their chance of adhesion to mucous and their persistence in the gut. In any case, when probiotic are supplied to weaning pigs, the possible action of already present secretory IgA should be considered. This could also explain unfavourable results of the probiotic strategy.

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