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# THE INFLUENCE OF SOW COLOSTRUM TRYPSIN INHIBITOR ON THE IMMUNOGLOBULIN ABSORPTION IN NEWBORN PIGLETS

By

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THODE JENSEN, P. and K. B. PEDERSEN: *The influence of sow colostrum trypsin inhibitor on the immunoglobulin absorption in newborn piglets.* Acta vet. scand. 1982, *23*, 161—168. — The influence of sow colostrum trypsin inhibitor (SCTI) on the immunoglobulin absorption from the gut of 16 newborn colostrum-deprived piglets was investigated in a paired feeding experiment.

Three times at 1 h intervals the piglets were fed an experimental diet consisting of sow milk, purified swine serum immunoglobulins containing agglutinins against Bordetella bronchiseptica, and purified SCTI (diet I) or saline (diet II). The serum concentrations of IgG, IgM, IgA, and antibodies for B. bronchiseptica were measured by single radial immunodiffusion and by a tube agglutination procedure and used to evaluate the immunoglobulin absorption. Four and 6 h after the first experimental meal, blood samples from the piglets given SCTI in their diet had a generally higher level of IgG, IgA and agglutinins against B. bronchiseptica than blood samples from the piglets fed no SCTI. No real differences were found in the IgM levels. Although the piglets fed no SCTI all showed a considerable immunoglobulin absorption, the SCTI was found to have a statistically significant positive influence on the IgG and IgA absorption.

pig immunoglobulins; sow colostrum trypsin inhibitor; immunoglobulin absorption.

The specific sow colostrum trypsin inhibitor (SCTI) (*Laskowski et al.* 1957) has long been assumed to play a role for the newborn piglets in their absorption of undegrated colostral immunoglobulins during the first 24—36 h of life (cf. *Brambell* 1970, *Jensen* 1978 a).

In a previous investigation (*Jensen & Pedersen* 1979), the levels of IgG and IgA, but not IgM, in serum from sucking piglets were found to be dependent on both the immunoglobulin levels and the SCTI level in the maternal colostrum. The object of the

present study was to examine by an experimental procedure, whether the hypothesis about the biological function of SCTI could be confirmed.

## MATERIALS AND METHODS

### *Animals*

The investigation included 2 or 3 pairs of equally sized, colostrum-deprived piglets from each of 3 Danish Landrace sows. The piglets were removed from their mothers immediately after they were born and kept at 32°C until the experiments were finished 6—10 h later. The two piglets of each pair were numbered at random with an even and an uneven number.

### *Experimental diet*

The experimental diet consisted of: 10 parts of a pool of sow milk, collected 10—15 days after farrowing; 4 parts of a solution of swine serum gammaglobulin containing specific antibodies against B. bronchiseptica; 1 part of an SCTI solution (diet I) or saline (diet II).

The gammaglobulin solution was prepared by ammonium sulphate precipitation of serum from a sow injected twice with a commercial B. bronchiseptica vaccine (Bortelvac vet. 'Kitasato'). Twenty-two g of ammonium sulphate was added per 100 ml serum at 4°C; after centrifugation the precipitate was redissolved at 22°C in a small volume of saline, and finally dialysed against saline. SCTI was prepared from a pool of sow colostrum by the following modification of the method of *Kress et al.* (1971): The colostrum was centrifuged at 18000 × g for 1 h at 4°C, whereafter the fat layer and the sediment were discarded and an equal volume of 5 % trichloracetic acid added slowly to the liquid. After stirring for 2 h at room temperature, the precipitate was filtered on Whatman No. 50 filter paper, 60.3 g ammonium sulphate was added per 100 ml filtrate, and the mixture left at room temperature overnight. After centrifugation the precipitate was redissolved in a 0.1 mol/l tris-HCl, 0.2 mmol/l $CaCl_2$ buffer, pH 8.2, dialysed against the same buffer, and gel filtered on a 2.5× 40 cm column of Sephadex G-100 (Pharmacia, Sweden). Trypsin-inhibitor-containing fractions were pooled, concentrated, and dialysed against saline. The same pool of sow milk but different preparations of gammaglobulin and SCTI were used for all of the 3 litters. The concentrations of immunoglobulins and SCTI

Table 1. Concentrations of immunoglobulins (IgG, IgM, IgA) and sow colostrum trypsin inhibitor (SCTI), and antibody titres against B. bronchiseptica, in the experimental diets.

| Litter No. | Diet No. | IgG g/l | IgM g/l | IgA g/l | SCTI g/l | Anti-B. bronchis. titre |
|---|---|---|---|---|---|---|
| I | I | 28.80 | 2.59 | 4.53 | 1.0 | 1:1280 |
| I | II | 27.30 | 2.89 | 4.36 | 0.0 | 1:1280 |
| II | I | 34.01 | 2.39 | 6.98 | 1.0 | 1:1280 |
| II | II | 34.00 | 2.43 | 7.41 | 0.0 | 1:1280 |
| III | I | 40.07 | 1.89 | 7.62 | 1.0 | 1:1280 |
| III | II | 40.69 | 2.02 | 7.10 | 0.0 | 1:1280 |

in the diets, and the agglutinin titres against B. bronchiseptica are given in Table 1.

*Experimental procedure*

The experiment was carried out separately for each litter. The few-hour-old piglets were fed 15 ml of the experimental diet by stomach tube 3 times at intervals of 1 h. The piglets numbered 1,3 and 5 received diet I and the piglets numbered 2,4 and 6 received diet II. Blood samples for serum preparation were drawn from the anterior vena cava at the first feeding of the piglets and again 4 and 6 h later. The serum samples were stored at —20°C until analysed. The analysis comprised total IgG, IgM and IgA levels, as well as specific antibodies for B. bronchiseptica.

For each of the 2 treatment groups, equal amounts of the sera obtained 6 h after the beginning of an experiment were pooled and gel filtered on a 1.6×70 cm column of Ultrogel ACA-34 (LKB, Sweden) equilibrated with a 0.01 mol/l tris-acetate, 1.0 mol/l NaCl, 3 mmol/l EDTA, 15 mmol/l $NaN_3$ buffer, pH 7.4, after having been dialysed against the same buffer. All elution fractions were analysed for IgG, IgM and IgA.

*Immunochemical procedures*

IgG, IgA, IgM and SCTI concentrations were determined by single radial immunodiffusion (*Jensen* 1977, *Jensen & Pedersen* 1979). Immunoelectrophoresis was performed by the micromethod of *Scheidegger* (1955) using antisera prepared as described previously (*Jensen & Pedersen*). Antibody titres for B. bronchiseptica were determined as indicated by *Pedersen & Barfod* (1977).

*Statistical methods*

The treatment effects were compared by the paired t-test (immunochemical results) and the sign test (serological results) (*Snedecor & Cochran* 1967).

## RESULTS

In all the blood samples collected at the beginning of the experiment, IgG, IgA and IgM concentrations were below 0.05 mg per ml serum, and no sample contained detectable antibodies against B. bronchiseptica. Four and 6 h after the piglets were fed their first meal there was a remarkable rise in their serum immunoglobulin levels, and they all had measurable serum agglutinins against B. bronchiseptica. The highest levels were found after 6 h among the piglets given SCTI in the diet (I) (Table 2). Both after 4 and 6 h, serum levels of IgG and IgA, and of antibodies against B. bronchiseptica were generally higher in the piglets given SCTI than in those not given SCTI. The differences in IgM level appeared to be random.

Table 2. Immunoglobulins (IgG, IgM, IgA) and agglutinins against B. bronchiseptica in serum from piglets 4 h and 6 h after their first meal with SCTI (diet I) or without SCTI (diet II).

| Litter - Diet - Piglet | IgG (g/l) | | IgM (g/l) | | IgA (g/l) | | B. bronchiseptica (aggl. titre) | |
|---|---|---|---|---|---|---|---|---|
| | 4 h | 6 h | 4 h | 6 h | 4 h | 6 h | 4 h | 6 h |
| I-I-1 | 2.06 | 4.70 | 0.09 | 0.34 | 0.37 | 0.87 | 1:80 | 1:160 |
| I-II-2 | 2.68 | 3.12 | 0.24 | 0.34 | 0.46 | 0.53 | 1:80 | 1:80 |
| I-I-3 | 0.31 | 1.57 | < 0.05 | 0.07 | 0.09 | 0.27 | 1:20 | 1:80 |
| I-II-4 | 1.03 | 1.45 | 0.18 | 0.21 | 0.32 | 0.33 | 1:40 | 1:40 |
| I-I-5 | 2.71 | 3.35 | 0.25 | 0.35 | 0.47 | 0.58 | 1:160 | 1:160 |
| I-II-6 | 0.51 | 0.75 | 0.09 | 0.12 | 0.19 | 0.24 | 1:20 | 1:20 |
| II-I-1 | 2.55 | 4.08 | 0.22 | 0.32 | 0.61 | 0.76 | 1:80 | 1:160 |
| II-II-2 | 2.20 | 2.97 | 0.19 | 0.24 | 0.57 | 0.57 | 1:80 | 1:80 |
| II-I-3 | 3.63 | 6.20 | 0.27 | 0.45 | 0.89 | 1.12 | 1:160 | 1:160 |
| II-II-4 | 1.80 | 3.40 | 0.13 | 0.26 | 0.49 | 0.73 | 1:80 | 1:80 |
| III-I-1 | 5.12 | 7.54 | 0.18 | 0.28 | 0.74 | 0.96 | 1:160 | 1:160 |
| III-II-2 | 2.70 | 3.67 | 0.15 | 0.22 | 0.49 | 0.64 | 1:80 | 1:80 |
| III-I-3 | 6.83 | 7.63 | 0.25 | 0.32 | 1.03 | 1.03 | 1:160 | 1:160 |
| III-II-4 | 4.31 | 5.31 | 0.21 | 0.30 | 0.76 | 0.87 | 1:80 | 1:160 |
| III-I-5 | 5.11 | 6.59 | 0.21 | 0.30 | 0.84 | 0.97 | 1:160 | 1:160 |
| III-II-6 | 4.31 | 5.49 | 0.23 | 0.30 | 0.71 | 0.81 | 1:160 | 1:160 |
| t | 2.34 | 4.62 | 0.24 | 1.71 | 3.51 | 6.49 | $\chi^2 = 0.8$ | $\chi^2 = 4.17$ |
| P | < 0.1 | < 0.01 | — | < 0.2 | < 0.01 | < 0.001 | — | < 0.05 |

The elution profiles for the different serum immunoglobulins from the Ultrogel ACA-34 gel filtrations were the same for the 2 groups of piglets, indicating similar molecular weight of the serum immunoglobulins at 6 h after their first meal. By immunoelectrophoretic analysis of the pooled and concentrated fractions collected after the elution of the immunoglobulins, traces of IgG-fragments — indicated by a changed electrophoretic mobility —- were found in serum from the piglets fed inhibitor-free diet, but not from piglets given SCTI in their diet.

## DISCUSSION

As is the case with immunoglobulins from other mammalian species, also porcine colostral immunoglobulins are sensitive to different proteolytic enzymes, including trypsin, as shown by *Stone et al.* (1979). Therefore protease inhibitor in colostrum may have a positive influence on the acquisition of passive immunity by newborn piglets.

In the present feeding experiment, the purpose of which was to evaluate the specific influence of the SCTI on the immunoglobulin uptake in the blood of neonatal piglets, it was important to be sure to measure only intact immunoglobulins in the piglet sera. The radial immunodiffusion method used for immunoglobulin quantitations is considered to be very sensitive, but may be unable to differentiate between intact and partially degrated immunoglobulins. However, the gel filtration studies showed the exclusion limits for the different serum immunoglobulins to be similar for the SCTI-deprived and the SCTI-fed piglets, which indicates that the molecular size of the respective immunoglobulins was the same in the 2 groups of piglets. Furthermore, by immunoelectrophoresis of the pooled and concentrated gel filtration fractions eluted after the immunoglobulins, only traces of immunoglobulin fragments were found and only in the pool from piglets without SCTI in their diet. The IgG fragments absorbed are assumed to be rapidly excreted by the kidneys, resulting in a very low serum level (*Hardy* 1969, *Martinsson* 1973). Assuming that only intact immunoglobulins were measured in the piglet serum the fact that the highest immunoglobulin levels were found in serum from the group of piglets fed SCTI indicates a real positive effect of the inhibitor on the absorption of intact immunoglobulin from the gut.

The addition of ammonium sulphate precipitated serum immunoglobulins to 10—15-day sow milk secured an experimental diet with a composition with respect to immunoglobulins almost like that of sow colostrum (*Bourne & Curtis* 1973). The use of sow milk collected 10—15 days after farrowing ensured that the basic diet was without any specific SCTI activity (*Jensen & Pedersen* 1979). The IgG and SCTI concentrations used in the diets corresponded to what had previously been found in colostrum from Danish Landrace sows (*Jensen* 1978 b), whereas the IgA and IgM levels were a little lower.

In most investigations concerning the assumed protective effect of the SCTI against tryptic degradation of proteins in the intestine of neonatal piglets, use has been made of heterologous trypsin inhibitors (reviewed by *Jensen* 1978 a) or of a blocking system with an excess of trypsin added (*Carlsson et al.* 1980). Previously, in a descriptive study, it had been found that the trypsin inhibitor content in the first colostrum from sows had a positive influence on the serum levels of IgG and IgA, but not IgM, in their few-day-old sucking piglets (*Jensen & Pedersen* 1979). This effect of the inhibitor has been verified experimentally in the present investigation, in which the SCTI was found also to have a positive effect on the absorption of agglutinating antibodies against B. bronchiseptica.

Both in the present investigation and in the study by *Carlsson et al.* piglets were found to absorb considerable amounts of intact immunoglobulins even if their diet was completely devoid of SCTI activity. Apparently the piglets fed the highest level of immunoglobulins had the relatively highest SCTI-independent immunoglobulin absorption, cf. Table 2. In an experiment with newborn colostrum-deprived piglets *Werhahn et al.* (1981) found the absorption of purified swine IgG to be directly proportional to the amount administered. One conclusion of these investigations may be that the SCTI has its greatest biological importance for piglets fed only small amounts of colostrum.

The consistent results of the different investigations concerning the function of SCTI permit the conclusion that a main biological function of the specific SCTI is to protect the colostrum immunoglobulins against proteolytic degradation in the guts of the piglets during the period when transfer of immunoglobulins from mother to offspring is taking place.

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SAMMENDRAG

*Betydningen af den specifikke so-kolostrum trypsin-inhibitor for immunglobulinabsorptionen hos nyfødte grise.*

Betydningen af den specifikke so-kolostrum trypsin-inhibitor (SCTI) for immunglobulinabsorptionen fra tarmen hos 16 nyfødte grise, der ikke havde haft adgang til at die, blev undersøgt ved et parvis opstillet fodringseksperiment.

Tre gange med 1 times mellemrum fik grisene en forsøgsdiæt bestående af somælk, oprenset svineserum immunglobulin indeholdende antistoffer mod Bordetella bronchiseptica og renset SCTI (diæt I) eller fysiologisk saltvand (diæt II). Serumkoncentrationerne af IgG, IgM, IgA og B. bronchiseptica antistoffer blev målt ved henholdsvis enkel radial immunodiffusion og agglutination i glas. Koncentrationerne blev anvendt til at vurdere immunglobulinabsorptionen. De grise, der fik SCTI, havde gennemgående et højere indhold af IgG, IgA og agglutininer i blodet 4 og 6 timer efter første fodring end de grise, som ikke fik SCTI. Der blev ikke fundet egentlige forskelle i IgM niveauerne. Selvom alle grise, der ikke fik SCTI, fremviste en betydelig immunglobulinabsorption, havde SCTI dog en statistisk signifikant positiv indflydelse på IgG og IgA absorption.

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