# The effect of colostral immunoglobulin supplement on the passive immunity, growth and health of neonatal calves

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NOUSIAINEN, J.[1], KORHONEN, H.[2], SYVÄOJA, E.-L.[2], SAVOLAINEN, S.[3], SALONIEMI, H.[3] & JALONEN, H.[4] 1994. **The effect of colostral immunoglobulin supplement on the passive immunity, growth and health of neonatal calves**. Agricultural Science in Finland 3: 421–428. ([1] Farm Services, Valio Ltd, P.O. Box 390, FIN-00101 Helsinki, Finland, [2] Research and Development, Valio Ltd, P.O. Box 390, FIN-00101 Helsinki, Finland, [3] College of Veterinary Medicine, Department of Animal Hygiene, P.O. Box 6, FIN-00581 Helsinki, Finland, and [4] Valio Bioproducts Ltd, Biocity, Tykistökatu 6, FIN-20520 Turku, Finland.)

Neonatal dairy calves were randomly allotted to three colostrum feeding regimens with increasing intakes of immunoglobulins (Ig) on the first day of life. The control group was fed one litre of pooled colostrum (Ig intake 19.5 g). In two experimental groups, the pooled colostrum was supplemented with 0.5 or 1.5 litres of commercial Ig-concentrate, giving a total Ig intake of 52.7 and 119.0 g, respectively. Serum IgG, IgM and IgA levels increased linearly ($p < 0.001$) on day 2 *post partum* with the increasing Ig intake. The calculated mean Ig-absorption rate was 61% and decreased linearly for IgM ($p = 0.051$) and IgG ($p = 0.078$) with increasing Ig intake. At the highest Ig intake, serum IgG remained above 10 g/l during 30 days *post partum*. In the experimental groups, serum IgM and IgA decreased sharply during the first week of life and were relatively constant thereafter. In the control group, however, there was an increase in serum IgM after one week *post partum*, perhaps due to the *in situ* production of Ig. With the increasing Ig intake there was a small and non-significant tendency for better live weight gain ($p = 0.286$) and a lower incidence of diarrhoea ($p = 0.421$) during the first four weeks of life. It is concluded that the Ig-product tested is well absorbed during 24 hours *post partum* and it can be used either as a supplement to maternal colostrum when its quality is poor, or as a substitute when colostrum is not available.

Key words: Ig supplement, immunoglobulin absorption, performance, diarrhoea

## Introduction

Colostral immunoglobulins (Ig) provide primary protection against infections in newborn calves, since the bovine placenta does not allow transfer of macromolecules in significant amounts (LARSON et al. 1980). There is, however, a marked variation in the Ig level of the first colostrum, possibly owing to the length of the dry period, number of lactations, feeding and other management factors of cows (FLEENOR and SCOTT 1981, KRUSE 1970a). Hence, quite many of the neonatal calves may be agammaglobulinaemic (e.g. BRIGNOLE and SCOTT 1980).

It has been suggested that newborn calves should have a total minimum intake of 80–100 grams of Ig (equals 2–3 litres of good quality colostrum) during the first 24 hours *post partum*

(KRUSE 1970b, SCOTT et al. 1979c). After this period, the gut epithelium closes, and Ig ingested by the calf may have only local importance against pathogens. HANCOCK (1985) reported an increased risk of mortality in calves with serum Ig levels below 2.5 g/l, but the occurence of diarrhoea may already increase at levels below 10 g/l. In a recent Swedish field study (VIRING et al. 1993), the mean serum Ig level in 7-day-old calves was 5.9 g/l and slightly less (5.3 g/l) in diarrhoeic calves. The peak Ig level was detected in samples taken during the day 2 *post partum*, averaging 8.5 g/l.

Since it is clear that the colostral Ig-concentration and the amount and time of its ingestion affect the level of circulating antibodies in the serum of calves and consequently their passive protection against diseases (SCOTT et al. 1979a-c), many artificial rearing methods have been studied. For example, frozen bulk colostrum (BESSER et al. 1991), pooled colostrum powder (ZAREMBA et al. 1993) and purified Ig powder made from cheese whey (FIEMS et al. 1989) or bovine blood serum (TODD et al. 1993) have been used as a partial or total supplement to dams' colostrum. However, little has been published about the absorption of different Ig classes from the different types of colostral supplement and their effect on the performance of neonatal calves has varied rather widely.

This study was designed to evaluate the effect of a commercial Ig preparation (Ig-C) given to supplement low-quality colostrum on the absorption of immunoglobulins, serum Ig level, growth and the occurrence of diarrhoea in neonatal calves.

## Material and methods

### Diets and animals

Twenty-one calves were divided at random into three groups, seven calves in each, and given one of the three feeding regimens (Table 1). Instead of dams' colostrum, the calves in the first group (control) were fed whole milk (WM) and pooled colostrum (PC). The other two groups were fed WM and PC and a commercial Ig concentrate (Ig-C) to give increasing controlled amounts of Ig from the first and second feeding in a constant volume (treat1 and treat2). In all groups the calves were first fed immediately *post partum* and the second time 8–12 hours later. In the control group, the Ig intake (about 20 g) represented a level at which calves ingest low-quality colostrum, in the treat1 group (about 50 g) a level which may be minimally acceptable, and in the treat2 group (>100 g) a level which may be regarded as safe.

WM was normal pooled milk from the experimental dairy herd. PC was collected from dairy farms in central and northeastern parts of Finland and was based on 1–5 milkings *post partum*. Colostrum was frozen on the farms, transported to a dairy factory, thawed, pooled and defatted by dairy separator. For usage in the feeding trial, PC was packed in 0.5-litre portions and frozen. Ig-C was commercially concentrated from PC by Valio Bioproducts Ltd., Turku, Finland. Casein was removed from PC by acid precipitation and the resulting whey was ultrafiltrated. The ultrafiltration retentate (Ig-C) was packed in 0.2-litre portions, sterilized (gamma-radiation, minimum 25 kGy) and frozen.

On days 4–56 of life all the calves were fed similarly with WM (10% of live weight), hay *(ad lib)* and commercial concentrate *(ad lib)*. The live weights of the calves were recorded immediately after birth and weekly thereafter until the age of 8 weeks. The faecal consistency was assessed daily during 28 days *post partum* on the following scale: hard (0), normal (1), soft (2) and diarrhoea (3).

### Sampling and analyses

Three separate samples of PC and Ig-C were taken from the respective lots used in the feeding trial. Blood samples were drawn from *vena jugularis* with dry vacuum tubes at 2, 7, 14 and 30 days of age. The samples were allowed to stand at room temperature for 30 min and serum was then separated by centrifugation (10 min, 2000 r/

Table 1. Feeding regimen during first four days of life.

| Treatment | First feding | Second feeding | Days 2–4 | Ig intake#) |
|---|---|---|---|---|
| Control | 1.00 l WM<br>0.50 l PC | 1.00 l WM<br>0.50 l PC | 3 l WM + PC-mixture*) | 19.5 |
| Treat1 | 0.50 l WM<br>0.50 l PC<br>0.50 l Ig-C | 1.00 l WM<br>0.50 l PC | 3 l WM + PC-mixture*) | 52.7 |
| Treat2 | 0.25 l WM<br>0.50 l PC<br>0.75 l Ig-C | 0.25 l WM<br>0.50 l PC<br>0.75 l Ig-C | 3 l WM + PC-mixture*) | 119.0 |

PC = pooled colostrum; WM = whole milk; Ig-C = colostral Ig concentrate.
*) Gradually decreasing proportion of PC (similar in all groups).
#) Total intake from first and second feeding.

min, WIFUG model X-1, Sweden) and stored at –20°C for analysis for IgG, IgM and IgA. The concentrations of different Ig classes in PC, Ig-C and serum were analysed with radial immunodiffusion assay using commercial kits (Serotec, UK). The estimated coefficients of absorption of the Ig classes were calculated as the ratio between the serum pool on day 2 to total intake, assuming the serum volume to be 7% of the birth weight (see SCOTT and MENEFEE 1978).

### Statistical methods

Serum Ig data on day 2, Ig absorption, live weights and growth were analysed with the regression analysis (Ig levels not equally spaced) with the following model $Y_{ijk} = \mu + \alpha T(l)_i + \beta T(q)_j + \varepsilon_{ijk}$ (1), where T(l) and T(q) represent the linear and quadratic effects of increasing Ig intake, respectively, and $\varepsilon_{ijk}$ deviations from the regression. In addition, the mean serum Ig levels and the treatment*age interaction was analysed with the split plot model $Y_{ijklm} = \mu + \alpha T(l)_i + \beta T(q)_j + \varepsilon_{ijk} + A_l + TA_{ijl} + \varepsilon_{ijklm}$ (2), where T(l), T(q) and $\varepsilon_{ijk}$ are as in the model (1), A is the effect of the age of the calves and $\varepsilon_{ijklm}$ is the sub-plot error. The calculations were made with the SURVO statistical program (MUSTONEN 1987). Faecal consistency observations were analysed with the $\chi^2$ test according to SNEDEGOR and COCHRAN (1967).

## Results

The concentrations of IgG, IgM and IgA in PC were 17.4, 1.9, and 0.2 g/l and in Ig-C 55.8, 9.2 and 1.3 g/l, respectively. The calculated total Ig intakes from the first two feedings were 19.5, 52.7 and 119.0 g for the control, treat1 and treat2 groups, respectively (Table 1).

The estimated absorption coefficiency of IgM and IgA decreased linearly with increasing intakes ($p = 0.051$ and $p = 0.078$, respectively, Table 2). A similar tendency was noted for IgG, but due to the high variation at low Ig intakes the trend was not significant ($p = 0.221$).

Increasing the Ig intake of the calves with Ig-C resulted in a linear response ($p < 0.001$) to serum IgG, IgM and IgA concentrations just after the absorption period on day 2 (Table 2). Serum IgG was above 10 g/l between days 2 and 30 with the highest Ig intake (Treat2, Figure 1). In the control and treat1 groups the minimum serum IgG was noted on days 7 and 14, respectively, increasing thereafter. The serum IgM and IgA concentrations declined sharply during the first week *post partum* at both levels of the Ig C-intake (Figures 2 and 3) being fairly constant from then onwards. In the control group, however, the concentration of IgM increased steadily after day 7, giving evidence of *in situ* production of IgM. For all Ig classes the

Table 2. Effect of the Ig concentrate on serum Ig levels and estimated absorption in treated calves between ages 2–30 days.

| Item | Control | Treat1 | Treat2 | SEM 18 df | Significance L | Q |
|---|---|---|---|---|---|---|
| Absorption, % | | | | | | |
| IgG | 68.8 | 57.5 | 45.4 | 7.42 | 0.221 | 0.828 |
| IgM | 73.2 | 52.1 | 42.4 | 5.66 | 0.051 | 0.389 |
| IgA | 87.9 | 71.9 | 50.1 | 8.25 | 0.078 | 0.850 |
| IgG, g/l | | | | | | |
| 2 days | 4.2 | 8.2 | 15.1 | 0.84 | < 0.001 | 0.832 |
| mean | 4.8 | 6.8 | 12.3 | 1.24 | < 0.001 | 0.696 |
| IgM, mg/l | | | | | | |
| 2 days | 483.4 | 1092.1 | 2182.9 | 102.95 | < 0.001 | 0.853 |
| mean | 502.1 | 586.8 | 959.6 | 79.40 | < 0.001 | 0.439 |
| IgA, mg/l | | | | | | |
| 2 days | 60.1 | 193.9 | 360.0 | 21.52 | < 0.001 | 0.478 |
| mean | 17.3 | 61.2 | 111.8 | 11.66 | < 0.001 | 0.337 |

Treatments on first day after calving: Control = 1.0 l pooled colostrum (PC), Treat1 = 1.0 l PM plus 0.5 l Ig concentrate (Ig-C), Treat2 = 1.0 l PC plus 1.5 l Ig-C.
Total Ig intake from first and second feeding: Control 19.5 g, Treat1 52.7 g and Treat2 119 g.
Significance: L = linear response to Ig intake; Q = quadratic response to Ig intake. Treatment*age interaction for mean IgG, IgM and IgA was significant ($p < 0.001$, see also figures 1–3). SEM = standard error of mean.

treatment*age interaction was highly significant ($p < 0.001$, Table 2, Figures 1–3).

Live weights (LW) and the LW gain of the calves are presented in Table 3. The responses of live weight and live weight gain to increasing amounts of Ig-C were non-significant ($p > 0.05$). There was a tendency for better LW gain during the first 28 days of life in calves treated with Ig-C but between 28 and 56 days the differences between the groups were small.

The distribution of faecal consistency observations is presented in Table 4. With increasing Ig intake there was a steady but non-significant ($p = 0.421$) tendency for a lower incidence of diarrhoea during the first month of life.

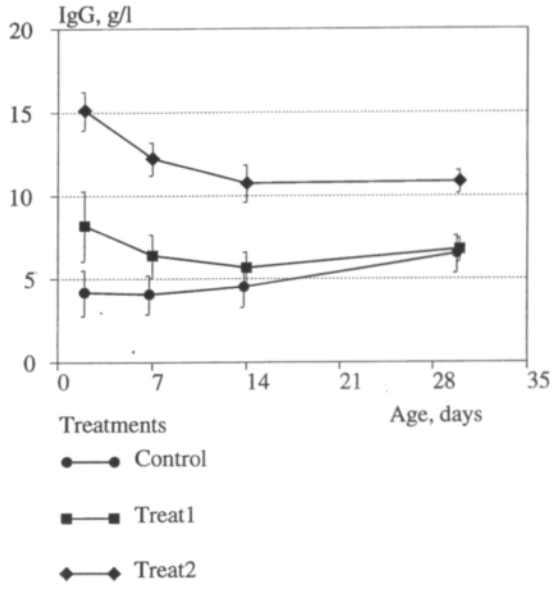


Fig. 1. Effect of Ig concentrate on IgG-level (mean ± S.E.) in serum of neonatal calves.

## Discussion

This study was designed to evaluate the efficacy of a commercial Ig preparation (Ig-C) as a sup-

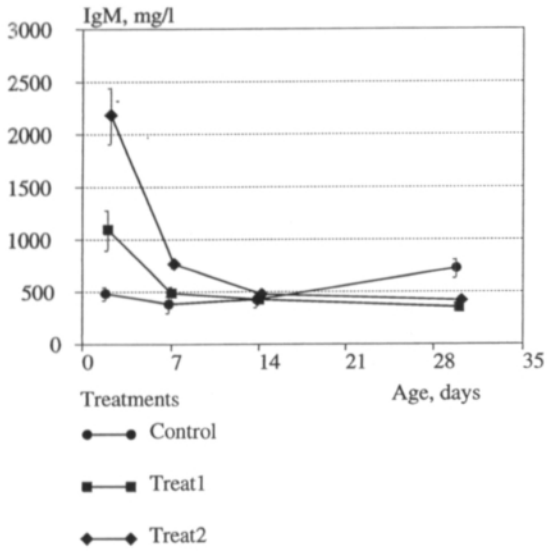


Fig. 2. Effect of Ig-concentrate on IgM-level (mean ± S.E.) in serum of neonatal calves.

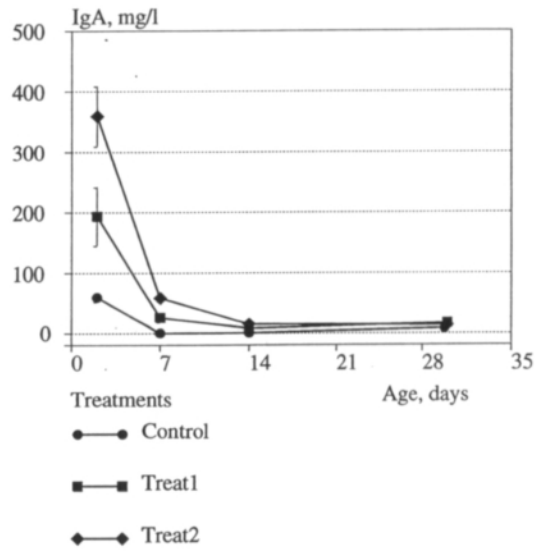


Fig. 3. Effect of Ig-concentrate on IgA-level (mean ± S.E.) in serum of neonatal calves.

plement to low quality colostrum. According to the results, the dose response of the peak serum IgG, IgM and IgA on day 2 to Ig-C seems to be linear (Table 2) when mixed with pooled colostrum having a relatively low Ig content. In agreement with this study PETRIE (1984) found that the serum Ig level in calves increased linearly with increasing Ig concentration. SCOTT and FELLAH (1983), who fed calves different levels of Ig by artificially varying the Ig concentration of colostrum, noted a linear increase in serum IgG and IgA; in contrast to our findings, however, serum IgM concentrations had a quadratic response. In an earlier study, SCOTT and MENEFEE (1978) found that the coefficient of absorption for IgM was decreased, and was constant for IgG and IgA when amount ingested increased from 50 to 400 g. BESSER et al. (1991) calculated a quadratic response

Table 3. Effect of the Ig concentrate on live weight and live weight gain in treated calves between ages 1 and 56 days.

| Item | Control | Treat1 | Treat2 | SEM 18 df | Significance L | Q |
|---|---|---|---|---|---|---|
| Live weight, kg | | | | | | |
| 1 day | 40.3 | 42.8 | 43.3 | 0.97 | 0.262 | 0.503 |
| 28 days | 49.0 | 52.4 | 54.0 | 1.22 | 0.133 | 0.527 |
| 56 days | 71.3 | 72.9 | 76.0 | 1.76 | 0.286 | 0.985 |
| Weight gain, g/d | | | | | | |
| 0–28 days | 309 | 343 | 381 | 18.6 | 0.286 | 0.809 |
| 28–56 days | 796 | 766 | 785 | 31.9 | 0.931 | 0.695 |
| 0–56 days | 553 | 550 | 583 | 22.4 | 0.546 | 0.796 |

Significance: L = linear response to Ig intake; Q = quadratic response to Ig intake.

Table 4. Effect of the Ig concentrate on occurrence of diarrhoea and soft faeces in treated calves between ages of 1 and 28 days.

| Item | Control | | Treat1 | | Treat2 | | Signficance |
|---|---|---|---|---|---|---|---|
| | f | % | f | % | f | % | |
| Distribution of observations | | | | | | | |
| – normal | 22.3 | (80) | 24.6 | (88) | 25.7 | (92) | |
| – soft | 3.3 | (12) | 3.0 | (11) | 1.7 | (6) | |
| – diarrhoea | 2.3 | (8) | 0.4 | (1) | 0.6 | (2) | |
| – soft + diarrhoea | 5.6 | (20) | 3.4 | (12) | 2.3 | (8) | 0.421 |

For treatments, see Tables 1 and 2. Faecal consistency was assessed on the following scale; 0 = hard, 1 = normal, 2 = soft, 3 = diarrhoea.
f = frequency of observations, mean per animal.

for serum IgG1 when the amount ingested increased from 20 to 300 g (constant volume of 2.84 l). The reason for the variable mode of absorption in single experiments might be the volume range of colostrum fed to calves and the method of varying the Ig intake. Here, the maximum total Ig intake was about 120 g during 24 h *post partum*, which is far lower than in the trials mentioned above (SCOTT and MENEFEE 1978, BESSER et al. 1991). In addition to the amount fed, the mode of absorption of Ig depends on the volume fed and the time of feeding (SCOTT and FELLAH 1983). Nevertheless, it may be reasonable to suggest that the mode of the peak serum Ig response is curvlinear at least with very high intakes, since the passive transfer system (pinocytosis) from the gut to the bloodstream may be saturated.

Our results suggest a linearly decreasing absorption efficiency of IgM and IgA with increasing intake. The decrease was 0.29 and 0.37 percentage units per 1 g increase of Ig intake for IgM and IgA, respectively. The mean coefficients agree well with those presented by SCOTT and MENEFEE (1978), although they noted selective absorption for IgM only with an increasing concentration in the colostrum. It is unclear wether Ig's were absorbed better from PC than from Ig-C, which was in fact processed from PC. First, increasing the Ig intake obviously lowers the absorption efficiency, and second, the present figures for absorption efficiency may be biased at low intakes, due to either analytical errors or the possibility of placental transfer of Ig. The rough regression estimates of pre-colostral serum values for IgG, IgM and IgA were 2.2, 0.2 and 0.02 g/l, respectively. In agreement with these figures, ZAREMBA et al. (1993) measured the serum IgG level between 1.2 and 1.4 g/l after birth before ingestion of colostrum. On the other hand, the pre-colostral value may reflect a genuine immunogloblin synthesis by the foetal calf during pregnancy owing to antigenic stimulation as speculated by JENSEN (1978). These points need further investigation, since placental transfer or the *in situ* production before parturition is uncertain (see e.g. LARSON et al. 1980).

In general, the serum Ig level in calves decreases after birth, being lowest 3–4 weeks *post partum* (LOGAN 1974, JENSEN 1978). In the present trial, especially IgM and IgA, but not IgG, were rapidly eliminated from the blood. The mean biological half life of IgM and IgA were 4.4 and 3.2 days, respectively, when calculated with regression between days 2 and 7. This is consistent with the results of JENSEN (1978). Owing to the significant treatment*age interaction in the serum Ig levels, we agree with LOGAN (1974) that in hypogammaglobunaemic calves *in situ* Ig syn-

thesis may begin as early as one week *post partum*. This is evident for IgM (Figure 2), but the serum IgG-level also tended to increase between days 7 and 30 in the calves with the lowest Ig intake (Figure 1). However, the earlier *in situ* Ig synthesis does not compensate for adequate colostrum intake in practice.

A number of authors have reported a close relationship between the Ig level in serum and the performance of calves (see HANCOCK 1985 and STAAK 1992). The data reported here suggest a slightly improving weight gain and less diarrhoea during the first month with increasing Ig intake (r = 0.35). None of the calves died or suffered severe health problems, although the serum Ig remained below the expected boundary level (5–10 g/l) in the control and treat1 groups. This may reflect good management methods and low infection pressure on the experimental farm, since high passive immunity *per se* does not necessarily improve performance. Immunoglobulins from a pooled colostrum source, as in this study (Ig-C), may not be as effective against antigens on a certain farm as those from the dam. On the other hand, pooled colostrum may be beneficial for transported calves, because it evidently contains antibodies against a variety of microbial antigens. To obtain a better understanding of this question, the specific antibody titres for Ig-C should be studied.

In the trials discussed by HALLIDAY (1980), the relationship between growth and the Ig level in blood was closer in suckling lambs than in dairy calves. The weight gain of lambs was closely related to the ability of the dam to produce milk. In other words, not only serum Ig level, but also energy and protein intake may partly explain the weak relationship between passive immunity and growth in some trials. In many colostrum supplementation trials (e.g. ZAREMBA et al. 1993, FIEMS et al. 1989, TODD et al. 1993), the response of growth and health status of calves to Ig supplements has been weak. Inadequate dosages, time of feeding or origin of the supplement may explain some of these results.

In conclusion, the concentrated Ig product tested here (Ig-C) caused a linear increase in the serum Ig level of dairy calves. About 0.5–1.0 litres of the product is needed to reach a safe Ig level (10 g/l) in the serum. Ig-C can be used either as a supplement to poor-quality maternal colostrum, or as a substitute when no colostrum is available.

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SELOSTUS

## Ternimaitoa täydentävän immunoglobuliini-tiivisteen vaikutus vastasyntyneiden vasikoiden immuniteettiin, kasvuun ja terveyteen

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Kokeessa selvitettiin kaupallisen ternimaidosta peräisin olevan Ig-tiivisteen vasta-aineiden imeytymistä ja vaikutusta vastasyntyneiden vasikoiden kasvuun ja ripulisuuteen. 21 vasikkaa jaettiin kolmeen seitsemän vasikan ryhmään, jotka saivat kasvavan määrän ternimaidon vasta-aineita kahdella ensimmäisellä juottokerralla. Kahdella ensimmäisellä juottokerralla kontrolliryhmä sai yhteisternimaitoa 1 l (Ig-saanti 19,5 g). Koeryhmissä vasikat saivat 1 l yhteisternimaidon lisäksi 0.5 tai 1.5 l kaupallista Ig-tiivistettä, jolloin vastaavasti Ig-saanti oli 52,7 ja 119,0 g. Maitomäärä tasattiin kaikille ryhmille samaksi täysmaidolla.

Seerumin IgG-, IgM- ja IgA-pitoisuudet nousivat suoraviivaisesti Ig-saannin lisääntyessä. Keskimääräinen laskennallinen vasta-aineiden imeytymistehokkuus oli 61 % ja se aleni lineaarisesti IgM:n ja IgA:n osalta. Eniten Ig-tiivistettä saaneessa ryhmässä seerumin IgG pysyi yli 10 g/l koko kokeen ajan, eli 30 päivää syntymästä. Molemmissa koeryhmissä seerumin IgM- ja IgA-tasot laskivat jyrkästi ensimmäisen elinviikon aikana, pysytellen sen jälkeen samalla tasolla aina 30 päivän ikään asti. Kontrolliryhmässä seerumin IgM-pitoisuus alkoi kuitenkin nousta ensimmäisen elinviikon jälkeen, johtuen mahdollisesti vasikan oman Ig-tuotannon alkamisesta. Ig-saannin noustessa vasikoiden kasvu parani ja ripulisuus väheni neljän ensimmäisen elinviikon, mutta erot eivät olleet tilastollisesti merkitseviä.

Tulokset osoittivat, että kokeessa tutkitun Ig-tiivisteen vasta-aineet imeytyvät hyvin 24 tunnin kuluessa syntymästä. Tuotetta voidaan käyttää vasikoille ensi sijaisesti emän ternimaidon lisäkkeenä, kun sen laatu on huono, tai korvikkeena kun sitä ei ole saatavilla.