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## Effect of Purified Immunoglobulins or Pooled Colostrum on Performance of Rearing Calves\*

L.O. FIEMS<sup>1</sup>, E. VANOPDENBOSCH<sup>2</sup>, Ch. V. BOUCQUE<sup>1</sup>, Y. VANCOILLIE<sup>3\*\*</sup> and B.G. COTTYN<sup>1</sup>

<sup>1</sup>National Institute for Animal Nutrition, Agricultural Research Centre-Ghent, Scheldeweg 68, 9231 Melle-Gontrode (Belgium)

<sup>2</sup>National Institute for Veterinary Research, Groeselenberg 99, 1180 Brussels (Belgium)

<sup>3</sup>Faculty of Agricultural Sciences, State University Ghent, Coupure Links 655, 9000 Ghent (Belgium)

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### ABSTRACT

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Fifty Belgian white-blue male calves, purchased from the market at about 10 days of age, were divided into three groups. The basic diet was the same for all calves, and consisted of a restricted amount of commercial milk replacer containing 50 ppm zinc bacitracin and 20 pm virginiamycin, calf starter and grass hay. Weaning occurred when daily starter intake over 7 consecutive days averaged 0.5 kg. Maximum starter intake was restricted to 3 kg day<sup>-1</sup>, and grass hay was fed ad libitum. The experiment lasted 20 weeks. Group 1 (control) received the basic diet. Groups 2 and 3 were supplemented with either 2 g purified immunoglobulins (Ig) or 25-ml pooled colostrum (CO) twice daily, via the milk replacer as used in Group 1.

Neither Ig nor CO exerted a positive effect on growth rate and feed efficiency. Six calves died: two, one and three in Groups 1, 2 and 3, respectively. Their serum IG content was significantly lower than that of the surviving animals.

Mechanisms explaining the lack of any protective effect are discussed. It is supposed that the main reason was due to the gap between the CO feeding at birth and the onset of the administration of Ig or CO at the start of the experiment. At that time, 31 of the 50 calves excreted rota- and/or coronaviruses.

### INTRODUCTION

The amount of colostrum and age at first feeding are the two most important factors which determine immunoglobulin concentration in the serum of neo-

\*Communication No. 715 of the National Institute for Animal Nutrition, Melle-Gontrode, Belgium.

\*\*Present address: Comelco International N.V., Keizerslaan 24, 1000 Brussels, Belgium.

natal calves (Stott et al., 1979a,b). When inadequate amounts or no colostrum are fed, poor gain and high morbidity and mortality may be expected (Nocek et al., 1984).

Both rotaviruses and coronaviruses are frequently associated with diarrhoea in newborn calves (Saif and Smith, 1985). According to Snodgrass and Wells (1978), antibodies in the blood serum appear to be of less value in the protection against these viruses. This may be a consequence of the finite half life of immunoglobulins in the intestine and the blood. The protective effect of early colostrum administration is gradually lost reaching a low level in the calf after about 7 days (Logan, 1983).

Rota- and coronaviruses infect and destroy villous epithelial cells and result in villous atrophy, which leads to malabsorption. Intestinal virus infection may retard growth (Woode, 1978). Therefore, an effective protection can be provided by continued feeding of small amounts of colostrum as part of the diet.

Separation technologies are currently available, so that minor whey components, containing immunoglobulins, can be provided for livestock production. The objective of this trial was to study the effect of feeding immunoglobulins during the initial 3 weeks of the rearing period on the performances of 10-day-old calves, taking into account that duration and amount of colostrum administration before purchasing were unknown. Consequently, the circumstances of the trial were a good reflection of the practical situation.

#### MATERIALS AND METHODS

Fifty Belgian white-blue male calves, purchased from the market at about 10 days of age, were assigned to one of three treatment groups based on live weight and conformation. The same commercial milk replacer, calf starter and grass hay were fed similarly to the individually penned calves. Calves of the control group (1) received the basic diet. Calves of Groups 2 and 3 received either 2 g immunoglobulins (Ig) or 25 ml pooled colostrum (CO) twice daily, respectively, via the milk replacer during the initial 3 weeks. Milk replacer was composed of 60% skim milk powder in order to obtain an EEC subsidy for feed manufacturers, and contained 50 ppm zinc bacitracin and 20 ppm virginiamycin. Ig were isolated from whey by a dairy factory via column chromatography. Colostrum was pooled milk from the first six milkings post-partum. Ig content averaged 44 mg ml<sup>-1</sup>. Calves were weaned when daily starter consumption averaged 0.5 kg over 7 consecutive days. Maximum starter allowance amounted to 3 kg per day, while grass hay was offered ad libitum. Chemical composition of the feeds and net energy content is given in Table 1. Energy content of starter and hay was assessed as described by Cottyn et al. (1987).

At the start of the experiment and at monthly intervals, blood samples were collected to study the effect on immunoglobulin concentration. Serum Ig de-

TABLE 1

Chemical composition and energy content of the feeds

	Milk replacer	Calf starter	Grass hay
Dry matter (DM, g kg <sup>-1</sup> )	967	872	858
Composition of DM (g kg <sup>-1</sup> )			
Crude protein	280	173	128
Ether extract	146	18	15
Crude fibre	-	72	282
Ash	72	82	93
Net energy lactation (MJ kg <sup>-1</sup> DM)	10.47	7.30	5.85

termination was carried out using liquid chromatography (FPLC-Pharmacia apparatus) and calculated via an external Ig-standard solution.

During the initial 4 weeks, faecal samples were taken to detect excretion of corona- and rotaviruses and *Cryptosporidium*. Antigens were determined via immunodiffusion for rotavirus (Vanopdenbosch et al., 1978) and enzyme-linked immunosorbent assay for corona virus (Vanopdenbosch et al., 1985). The presence of *Cryptosporidium* was detected by the carbol fuchsin method (Peeters et al., 1982). At autopsy, viral antigens were evidenced by direct immunofluorescence in organ sections. Enterotoxigenic *Escherichia coli* K99 was detected using two methods: (1) indirect immunofluorescence (Lintermans and Pohl, 1984); (2) isolation on a Minca glucose medium after an incubation at 37°C for 24 h and identification by slide agglutination with K99 antiserum (Girardeau et al., 1979).

The experiment lasted 20 weeks. Effect on live-weight gain, feed intake, virus and *Cryptosporidium* excretion, diarrhoea and mortality was investigated.

## RESULTS AND DISCUSSION

Six calves died within the first 4 weeks of the experiment: two in Group 1, one in Group 2 and three in Group 3. Antigens found at autopsy are given in Table 2.

Serum immunoglobulin content of these calves was low in comparison with those in the calves which survived ( $P < 0.01$ ) (Table 3).

These data correspond with those reported by Brignole and Stott (1980), and emphasize the importance of colostrum for the newborn calf. Low serum Ig levels can be the consequence of a lack of CO feeding, an advanced age at first feeding (Stott et al., 1979a,b), or a failure to absorb Ig (Brignole and Stott, 1980).

TABLE 2

Antigens in carrions

Group	Calf	Organ	Antigen
1	1	Intestine	<i>E. coli</i> Y
		Lung	Parainfluenza 3
	2	Intestine	<i>E. coli</i> K99, Coronavirus
		Lung	Parainfluenza 3
2	3		Negative
3	4	Intestine	Coronavirus
		Spleen	<i>E. coli</i> Y
	5		Negative
	6	Intestine	Rotavirus

TABLE 3

Concentration of serum immunoglobulins at the start of the trial

	Calves which died	Calves which survived
Number	6 (12.0) <sup>1</sup>	44 (88.0)
Ig (mg ml <sup>-1</sup> )	7.06 <sup>a</sup> ± 1.89 <sup>2</sup>	16.90 <sup>b</sup> ± 1.13

<sup>a,b</sup>Values are significantly different ( $P < 0.01$ ).<sup>1</sup>Percent of calves.<sup>2</sup>Standard error of the mean.

TABLE 4

Evolution of serum immunoglobulin concentration<sup>1</sup> (mg ml<sup>-1</sup>)

Blood sampling	Control	Ig	CO
Start	16.6 ± 2.2	15.7 ± 1.8	14.7 ± 1.8
Day 35	15.4 ± 1.0	14.7 ± 1.0	15.9 ± 1.0
Day 72	19.1 ± 1.1	17.1 ± 1.0	16.4 ± 1.0
Day 94	17.4 ± 0.8	15.8 ± 1.0	16.0 ± 0.9
Day 139	16.2 ± 0.9	16.4 ± 1.1	14.0 ± 1.0

<sup>1</sup>No significant differences ( $P > 0.05$ ).

The serum immunoglobulin concentrations during the experiment are presented in Table 4.

At no time was there a significant difference in serum Ig concentrations. This meant that serum Ig level could not be affected by oral administration

some days post-partum. These findings confirm the fact that closure of intestinal permeability to colostrum Ig in the calf occurs spontaneously with age from 12 h post-partum (Stott et al., 1979a).

For 31 of the 50 calves, virus shedding took place from the first few days after arrival (Table 5).

During the first 3 weeks of the trial, virus and *Cryptosporidium* infection were observed in almost all calves of the three groups, without any difference in respect of the treatment (Table 6).

Duration and frequency of diarrhoea were not influenced by the treatment (Table 7). Forty-four of the 50 calves presented diarrhoea. In 27 of them, the diarrhoea lasted for 2-7 days and in 14 calves for > 7 days.

The effects of treatments on daily gain and weaning age are compiled in Table 8.

Average daily gain was not significantly affected during the first 6 weeks of the treatment, although some effect could be expected from Ig and CO within these initial weeks in view of the widespread virus excretion. From the seventh

TABLE 5

Virus excretion at arrival

Virus	1. Control (n = 17)	2. Immunoglobulins (n = 16)	3. Colostrum n = 17)
Coronavirus	11	5	5
Coronavirus + rotavirus	2	3	2
Rotavirus	-	-	3
Number of excreting calves	13/17	8/16	10/17

TABLE 6

Virus and *Cryptosporidium* excretion during the first 3 weeks

Virus and/or <i>Cryptosporidium</i>	1. Control (n = 17)	2. Immunoglobulins (n = 16)	3. Colostrum (n = 17)
Coronavirus	3	5	3
<i>Cryptosporidium</i>	-	1	-
Coronavirus + rotavirus	5	1	5
Coronavirus + <i>Cryptosporidium</i>	3	4	1
Coronavirus + rotavirus + <i>Cryptosporidium</i>	4	5	5
Number of excreting calves	15/17	16/16	14/17

TABLE 7

Occurrence of diarrhoea during the first 3 weeks

Duration of diarrhoea	Group 1, control (n=17)	Group 2, immunoglobulins (n=16)	Group 3, colostrum (n=17)
1 day	1	1	1
2-7 days	8	11	8
> 7 days	4	4	6
Number of diarrhoeic calves	13/17	16/16	15/17

TABLE 8

Effect of immunoglobulins and colostrum on live-weight gain and weaning age of white-blue male calves

	Group 1, control	Group 2, immunoglobulins	Group 3, colostrum
Number of calves	15	15	14
Initial weight (kg)	49.1 ± 1.9 <sup>1</sup>	49.1 ± 1.7	49.7 ± 1.7
Final weight (kg)	167.3 ± 3.6	166.3 ± 3.7	165.6 ± 4.0
Daily gain (kg)			
0- 6 weeks	0.45 ± 0.03	0.43 ± 0.04	0.45 ± 0.03
7-12 weeks	0.87 ± 0.03	0.85 ± 0.03	0.88 ± 0.03
13-20 weeks	1.12 ± 0.01	1.13 ± 0.03	1.08 ± 0.02
0-20 weeks	0.84 ± 0.02	0.84 ± 0.02	0.83 ± 0.02
Weaning age (days)	35.3 ± 1.6	36.1 ± 2.0	34.8 ± 2.0

<sup>1</sup>Standard error of the mean.

week, there was no impact on live-weight gain. During this period, a positive effect owing to the previous feeding of Ig and CO was hardly expected since the animal's own immune system begins to develop at about 3 weeks of age. For the 20 weeks of the experiment, daily live-weight gain averaged 0.84, 0.84 and 0.83 kg, respectively, for the three groups. There was no significant effect on weaning age. This is an indication that calf starter intake during the initial period of the experiment corresponded well between the three groups.

Mean daily intake of dry matter and net energy (NE<sub>1</sub>) was similar for all groups. Consequently, this was also true for feed efficiency (Table 9).

The similar feed conversion suggests that, if there was some malabsorption owing to villous atrophy by virus infection (Mebus et al., 1975), treatment effect on intestinal absorption of nutrients and energy was not different. Otherwise, the protective effect of Ig and CO was doubtful in these circumstances.

TABLE 9

Effect of immunoglobulins and colostrum on intake and feed efficiency

	Group 1, control	Group 2, immunoglobulins	Group 3, colostrum
Total feed intake (kg per calf)			
Milk replacer	17.0 ± 0.9 <sup>1</sup>	16.4 ± 0.9	16.6 ± 1.0
Calf starter	274.9 ± 5.8	276.5 ± 6.4	270.6 ± 7.6
Grass hay	82.4 ± 5.3	79.6 ± 6.1	74.5 ± 5.6
Mean daily intake kg <sup>-1</sup> W <sup>0.75</sup>			
DM (g)	69.6 ± 0.8	69.4 ± 1.2	67.5 ± 1.3
NE <sub>1</sub> (MJ)	0.50 ± 0.01	0.50 ± 0.1	0.48 ± 0.01
Feed conversion			
0-6 weeks: DM (kg)	1.88 ± 0.15	1.85 ± 0.11	1.83 ± 0.17
NE <sub>1</sub> (MJ)	15.85 ± 1.3	15.75 ± 1.1	15.60 ± 1.3
0-20 weeks: DM (kg)	2.77 ± 0.06	2.77 ± 0.04	2.73 ± 0.06
NE <sub>1</sub> (MJ)	19.8 ± 0.4	19.8 ± 0.2	19.5 ± 0.4

<sup>1</sup>Standard error of the mean.

The relationship between initial serum Ig concentration and performance was small, when the data of the 44 surviving calves were pooled. The correlation coefficient between the serum Ig level at the start, and daily gain during the initial 6 weeks and the complete experiment amounted to 0.223 and 0.302. For the same periods, the correlation coefficients between Ig level and energy conversion were -0.180 and -0.004. It is possible that the serum Ig concentration analysed at the start of the experiment was not a true indication of the concentration at Day 1 after birth because of the finite half life of Ig and the fact that the exact age of the calves at purchase was not known.

The protective activity of colostrum, milk or serum Ig administered during the first weeks of life against viral neonatal diarrhoea has been confirmed by several authors.

Snodgrass et al. (1980, 1982b) stated that diarrhoea started later, that the frequency, duration and severity of diarrhoea diminished markedly, and that body weight improved in calves fed during the first 2 weeks with 10% colostrum from rotavaccinated cows.

Snodgrass et al. (1982a), Snodgrass (1986a, b), Soulebot et al. (1983) and Bürki et al. (1986) observed good protection against viral diarrhoea following continued feeding of immune milk from vaccinated cows during the first weeks of life. Vanopdenbosch and Wellemans (1981) reported on a similar experiment in 24 problem herds, that the percentage of diarrhoea decreased from 79 to 27, the onset of diarrhoea was retarded from Day 4 until Day 9, the average



duration of diarrhoea decreased from 13 to 2 days and the mortality rate from 29% to 1%.

Theys et al. (1983) described similar results in an experiment with veal calves fed with milk replacer that contained serum from slaughtered calves hyperimmunized against rota- and coronavirus.

The exact mechanism explaining the discrepancy between the local prophylactic effect of Ig in the intestine, reported in the literature and the non-protective effect in our experiment is not clear. It is possible that the Ig supplementation was too small. An amount of 4 g Ig was fed daily to calves averaging 49.1 kg live-weight in our experiment. In an experiment with neonate pigs, which were removed from their dams at birth, 10 g Ig were fed  $\text{kg}^{-1}$  body weight on Day 1, and 2 g on Days 2–10 (Elliot et al., 1987). Survival in piglets in the test group was 78% vs. 39% in the control group. From birth to 21 days, the test group realized a 40% higher weight gain. Obviously, 0.5-g  $\gamma$ -globulin was not sufficient to affect survival in piglets (Varley et al., 1986).

It is also possible that the effectiveness of Ig was small in our experiment because antibodies did not correspond with antigens, or titres were not high enough. Since Ig were provided by a dairy factory and produced by large populations of cows, we suppose that Ig were active against most important strains of pathogens.

Another reason could be that the prophylactic effect failed to appear in this trial because of the largely extended infections. In such a situation, Ig may be degraded in the intestinal lumen by microorganisms. This mechanism was mentioned by James et al. (1976), but it is not clear whether bacteria and viruses behave similarly.

In our calves, 31 of 50 were excreting rota- and/or coronaviruses at the start of the trial (first faecal collection). Hence, it is possible that Ig administration started too late to exert any protective effect. In this context, Donnelly (1987) suggested that the local role of Ig appear to be prophylactic by preventing the establishment of new pathogens, but they may have little effect on pathogens which are already established. According to Logan and Pearson (1978), it is possible that Ig exert their protective function by forming an immunological barrier which prevents the adhesion at or the penetration into villi by enteropathogenic microorganisms.

Besides Ig, colostrum contains other non-specific inhibitory systems, such as lysozyme, lactoferrin, lactoperoxidase, xanthine oxidase, vitamin B<sub>12</sub> and folate protein binders (Reiter, 1978). Therefore, a broader protective role by CO than by Ig alone could be expected. However, CO was not of excellent quality, as it contained only 44 mg Ig  $\text{ml}^{-1}$  (Fleenor and Stott, 1980). This can be explained by the fact that CO from the first six milkings post-partum was pooled. Based on the data of Stott et al. (1981) for pooled CO of six milkings post-partum, an average Ig concentration of 28.6 mg  $\text{ml}^{-1}$  can be calculated. As a consequence, the daily Ig administration in Group 3 ( $\pm 2.2$  g) was lower

than in Group 2. Obviously, non-specific inhibitors in CO were not capable of making up for this lack of protection.

We suppose that the main reason for the failure of any protective effect of pure Ig or CO in these purchased calves is due to the gap between the CO feeding at birth and the start of the experiment, coupled with the fact that the calves were infected in the meantime. This necessitates the administration of pure Ig following up CO feeding after birth, or the continuation of feeding small amounts of CO.

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