

## Solid feed provision reduces fecal clostridial excretion in veal calves

*Het geven van vaste voeding aan witvleeskalveren vermindert de uitscheiding van clostridia in de mest*

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## ABSTRACT

Enterotoxemia is characterized by a highly fatal hemorrhagic enteritis in cattle, caused by *Clostridium perfringens*. Production systems with intensive feeding, such as the veal industry, are predisposed. The primary objective of this study was to determine the effect of solid feed provision on fecal *C. perfringens* excretion in veal calves. Ten Holstein Friesian bull veal calves were randomly assigned to one of two test diets. Group 1 received solely milk replacer twice daily, while group 2 received milk replacer and a maximum of 300g solid feed/day, consisting of a mixture of 30% barley, 30% corn, 30% hulled wheat and 10% chopped straw. The number of *C. perfringens* per g feces or fecal clostridial counts (FCC) were determined for all calves. Mean FCC were significantly lower in the calves fed milk replacer and solid feed, than in the calves fed solely milk replacer. Although the correlation between FCC and enterotoxemia risk remains to be determined, the provision of solid feed to veal calves reduced clostridial excretion, which might contribute to the prevention of this disease.

## SAMENVATTING

*Clostridium perfringens* geassocieerde enterotoxemie is een belangrijke oorzaak van sterfte bij kalveren in intensieve opfoksystemen. Het doel van deze studie was om het effect van vaste voeding te beoordelen op de uitscheiding van *C. perfringens* in de mest bij witvleeskalveren. Twee verschillende testdiëten werden at random toegewezen aan tien holsteinfriesian-witvleeskalveren. De kalveren in groep 1 werden uitsluitend gevoed met melkpoeder, terwijl kalveren in groep 2 zowel melkpoeder als een maximum van 300g vaste voeding per voederbeurt kregen. De vaste voeding bestond uit 30% gerst, 30% mais, 30% tarwe en 10% gehakseld stro. Vervolgens werd het aantal clostridia in de mest bepaald. Kalveren die zowel melkpoeder als vaste voeding kregen, scheidde significant minder clostridia uit in de mest dan kalveren die uitsluitend met melkpoeder werden gevoed. Er moet nog verder onderzocht worden of het verschaffen van vaste voeding aan witvleeskalveren een preventief effect heeft op enterotoxemie.

## SHORT COMMUNICATION

Enterotoxemia (hemorrhagic enteritis) is a highly fatal disease in cattle, caused by *Clostridium perfringens*. The disease predominantly affects calves in intensive production systems, such as the veal industry or feedlots (Griner and Bracken, 1953; Niilo

et al., 1974, Glock and Degroot, 1998; Muylaert et al., 2010). In Belgian blue veal calves, enterotoxemia accounts for 20% of the total mortality rate (5-7%) and occurs predominantly at the end of the production cycle when animals have a high economic value (Pardon et al., 2012). Given the high fatality rate and rapid course of the disease, control is mainly focused

on prevention. It is generally accepted that especially dietary factors, such as high protein diets are associated with the enterotoxemia risk (Lebrun et al., 2010). Therefore, dietary adaptations might help in the prevention of the disease. Traditionally, white veal calves are not fed any solid feed in order to maintain the desired pale meat color. In recent years, several studies have demonstrated beneficial effects of the provision of solid feed on ruminal development, daily growth and animal welfare, without negative effects on meat color (Suarez et al., 2007; Webb et al., 2013). Moreover, for welfare reasons, European legislation obliges a minimum provision of solid feed (European Council Directive 2008/119/EC). It is hitherto unclear what the consequences of solid feed provision are with respect to the risk of developing enterotoxemia. It is believed that fiber-rich feeds inhibit clostridial overgrowth, by altering the intestinal microbiota (Zhang et al., 2013). Thus, the hypothesis of the present study was that solid feed provision would reduce the fecal excretion of *C. perfringens* in veal calves.

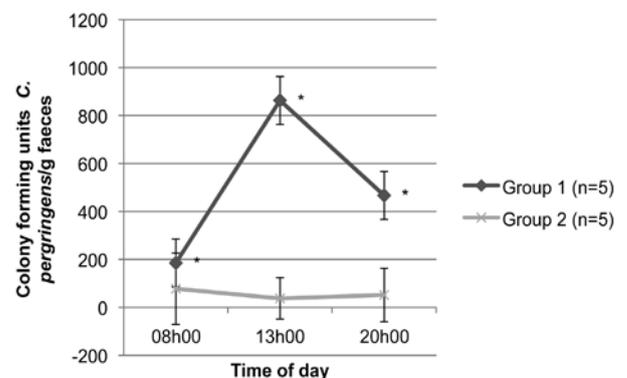
To determine the effect of solid feed provision on fecal *C. perfringens* excretion a cross-sectional study was conducted on a commercial white veal farm (5000 animals present). To determine a one log difference between test groups, with a standard deviation of 40 CFU (colony forming units)/g feces, 95% confidence and 80% power, a sample size of 5 animals per test group was required. All procedures were approved by the local ethical committee (EC2014/016). Ten male Holstein Friesian calves (mean body weight= 52kg; mean age=14 days) were randomly assigned to one of two test groups (group 1 and group 2) on the day of arrival at the fattening unit. All calves were kept on a slatted floor without bedding. Calves were not vaccinated against Clostridia. However, both groups received amoxicilline in the first ten days after arrival at the fattening unit as a preventive start-up treatment. Both groups were fed the same amount and concentration of a commercial milk replacer (Nil product, Vilatca nv, Geel, Belgium), twice daily. The milk replacer provision increased from 220g DM (dry matter)/day on arrival to 750g DM/day at the age of three months. The milk replacer contained 8% ash, 17.7% crude fat and 18% crude protein (CP) (whey, vegetal protein, no casein) per feeding. Group 2 received solid feed once daily in addition to the milk replacer, starting with 32g FP (fresh product)/feeding gradually increasing to 300g FP/feeding at the age of three months. The solid feed consisted of a mixture of whole grains (barley (30%), corn (30%), hulled wheat (30%)) and 10% chopped straw (ash 2.4%, CP 9.6%).

Calves were sampled at the age of three months. Fecal samples were taken from each calf three times per day (in the morning shortly after feeding around 8.00h, at midday halfway between feedings around 13.00h and in the evening shortly before feeding around 18.00h). All fecal samples were taken with rectal gloves directly from the rectum, and cooled to 4°C until processing within 24 hours. Each sample

was plated nine times (technical repeats). Quantification of *C. perfringens* was performed as follows. One gram of the fecal samples was suspended in 9 mL of PBS (phosphate buffered saline). Ten-fold dilutions were made in sterile PBS. Subsequently, for each dilution, six droplets of 20 µL were plated on Columbia agar (Oxoid, Basingstoke, UK) with 5% defibrinated sheep blood, containing 12 mg kanamycin sulphate and 30,000 U/l polymyxin B sulphate. Bacteria were allowed to grow overnight at 37°C in an anaerobic cabinet (Ruskinn Technology, Bridgend, UK) with 84% N<sub>2</sub>, 8%CO<sub>2</sub> and 8% H<sub>2</sub>. The identification of *C. perfringens* colonies was made on the basis of a dual hemolysis zone. The number of CFU of *C. perfringens* per g feces (= fecal clostridial count (FCC)) was calculated based on the recovered colonies on the plates. To determine the effect of the diet on the FCC, a linear mixed model with repeated measurements was built with clostridial count (in CFU/g feces) as outcome variable. For each calf, three observations were added, one at each time point (morning, midday and evening). Each observation consisted of the mean of nine FCC performed on each sample. The outcome variable was tested for normal distribution. Time (morning-midday or evening) was added as a repeated measurement and a variance components covariance structure was used. Calf was added as a random effect to account for clustering of samples within a calf. Feeding regime (Group 1 or 2) was added as a fixed factor. Significance was set at P<0.05. All analyses were performed in SAS 9.4 (SAS Institute Inc., Cary, NC), using the PROC MIXED procedure.

Mean±SD FCC was 505±1059 CFU/g feces and 56±119 CFU/g feces in groups 1 and 2, respectively. The effect of the test group was significant at every time point (P<0.001). The time effect (8.00h, 13.00h, 20.00h) was not significant (Figure 1).

The study of risk or protective factors for enterotoxemia is seriously hampered by the fact that this is a rare event disease (Lebrun et al., 2010). Unrealistically large sample sizes need to be taken into account



**Figure 1.** Evolution of the mean fecal clostridial counts (CFU/g feces) in veal calves receiving only milk replacer (group 1) or milk replacer and solid feed (group 2). Error bars represent the standard deviation; \* indicates a significant difference (P<0.001) between the two groups at that time.

when looking at mortality due to enterotoxemia as the outcome variable. Therefore, alternatively, the effect of solid feed provision on fecal *C. perfringens* excretion was determined. The authors want to stipulate that the observations made in this study refer to FCC. Evidencing a direct link with enterotoxemia was not the objective. Higher FCC suggests an increased clostridial load in the intestinal microbiota, which may contribute to a higher risk of *C. perfringens* induced enterotoxemia. However, there is no known FCC threshold that would result in enterotoxemia.

The present study showed that supplying solid feed decreases fecal clostridial excretion in veal calves. The mechanism behind this effect is not completely understood. Veal calves fed exclusively milk replacer only develop a rudimentary rumen (Suarez et al., 2007). In the veal calves offered solid feed, the limited amount of 300g per feeding even induced relevant ruminal development and an active ruminal function (Webb et al., 2013). This might lead to an intestinal environment less favorable for Clostridia, possibly favoring bacteria with a direct *C. perfringens* growth-inhibiting effect, such as for example *Bacillus subtilis* (Jeong and Kim, 2014). Fiber-rich feeds indeed do increase the proportion of *Bacillus spp.* in the ruminal microbiota, potentially inhibiting clostridial overgrowth (Zhang et al., 2013). Moreover, veal calves fed milk replacer and solid feed have an improved ruminal digestibility (Labussiere et al., 2009), potentially leading to a decreased availability of nutrients in the lower intestine, and thus leading to a lower FCC. Although the association between FCC and enterotoxemia risk remains to be determined in calves, the provision of solid feed to veal calves might be a useful preventive tool against enterotoxemia. This opens perspectives for further dietary management of the disease. It remains to be determined whether the composition of the solid feed can be optimized in order to reduce FCC even further.

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