Long term effects of probiotic dry cow formula on the gestation and colostrum quality of Holstein cows

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Enterococcus faecium SF68® (SF68®) is considered one of the best characterized probiotic active ingredient worldwide.

The strain has been isolated in Sweden from a healthy newborn baby in the sixties and has been demonstrated to have clinical benefits for humans (Wunderlich et al., 1989).

Furthermore, this probiotic strain is also effective in modifying the immune response of piglets, young dogs, mice and cats (Bakhshi et al., 2006; Benyacoub et al., 2005; Bybee et al., 2011; Scharek et al., 2005; Simpson et al., 2009).

Manufacturer: Cerbios-Pharma S.A. Switzerland
Cernivet® LBC ME10 is registered in EU for the use in:

- Calves up to 6 months,
- Chickens for fattening,
- Sows (2 weeks before farrowing and during lactation),
- Piglets up to 4 months and
- Pigs for fattening.

(http://www.cerbios.ch/l/biological-product/cernivet®-lbc-me10-and-cernivet®-lbc-me20-plus)
The application of probiotics to ruminant animals is relatively rare due to the presence of pre-gastric fermentative rumen.

Nevertheless, a novel route of probiotic application via teat canal directly into the mammary lumen of dairy animals has been developed for the prevention and treatment of mastitis (Ryan et al., 1998, 1999; Crispie et al., 2008; Klostermann et al., 2008; Beecher et al., Frola et al., 2012).
In our recent studies, a preparation of SF68 was used to substitute for the commercial antibiotic dry cow formula in Holstein cows and was found with extra benefits of enhancing the local innate immune function (Peng et al. 2013) and accelerating the involution process (Tiantong et al. 2014) of mammary gland during the acute phase of drying-off.

In the current study, the effects of applying probiotics SF68 as a dry
cow formula on the final stage of pregnancy and the subsequent
parturition and lactation of Holstein cows.
Cow selection and management

- Lactating, second parity Holstein cows at 24 - 56 months of age were raised at the University Farm of National Chung Hsing University (Taichung, Taiwan).
- Milk stasis was practiced when cows were reaching the final two months of pregnancy.
- Milk yield was less than 5 kg/day.
- The experimental procedures have been approved by the committee of Care and Use of Agriculture Animals of the university.
Experimental design

Drying-off

30 days

60 days

30 days

Colostrum sampling

Newborn Calf sampling

SF68 infusion

n=3

n=2

D 1

D 3

D 7

D 30

Parturition

MATERIALS AND METHODS
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- 2.5-ml of ultrasonicated SF68 ($2 \times 10^8$ cfu/ml)
- 2.5-ml of commercial dry-cow therapy

5-ml of commercial dry cow therapy
Cow analysis:
- Plasma hormone level
- Plasma total protein content and composition
- Plasma gelatinase level
- Blood leukocyte number and function

Colostrum analysis:
- Total protein content and composition
- Gelatinase level

Neonatal calves analysis:
- Plasma total protein content and composition
- Plasma gelatinase level
- Blood leukocyte number and function
Figure 1 Typical images of SDS-PAGE and gelatin zymography for the evaluation for cow plasma.
**Figure 2** Summarized results of two individual cows showing the levels of plasma hormones (solid lines) and the means (dotted lines). *

Mean value is significantly different from the corresponding mean of day 1. $P < 0.05$. 

**COWS RESULTS**
Figure 3 Summarized results of two individual cows showing the levels of plasma total protein content and compositions of gamma globulin and albumin (solid lines) and the means (dotted lines).
Figure 4 Summarized result of two individual cows showing the blood total leukocyte count and function, and plasma MMP-2 level (solid lines) and the means (dotted lines). * ,Mean value is significantly different form the corresponding mean of day 1. $P < 0.05$. 
COLOSTRUM RESULTS

Figure 5 Representative SDS-PAGE images of initial colostrum of two individual cows.
**Figure 6** Summarized result of cow showing the colostral total protein content, compositions of major colostral proteins for the control and SF68-infused udders of the two individual cows.
Figure 7 Summarized result of cow showing the colostral levels of MMP-2 and MMP-9 for the control and SF68-infused udders of the two individual cows.
Figure 8 Summarized result of two individual neonatal calves showing the blood total leukocyte count and function, plasma total protein content and composition of gamma-globulin and albumin, and plasma MMP-2 level.
The most prominent change observed in the current study in association with intramammary SF68 infusion is the elevation of MMP-2 level, both systemic and local mammary gland.

The presence of MMP in animal tissues and fluids has been used as supplementary information for diagnosing diseases monitoring organ remodeling, including horse arthritis (Jouglin et al., 2000), cow mastitis (Rabot et al., 2002) and different stages of mammary gland involution (Yu et al., 2012).

The higher MMP-2 level in plasma and colostrum is very likely related to some subclinical proinflammation in the body and mammary gland.
No change in most of the conventional physiological parameters of experimental cows during the nearly two-month period following SF68 infusion.

Regarded clinically normal of final stage of pregnancy, the parturition, the colostrum quality and the neonatal calves of Holstein cows after intramammary SF68 infusion.

Higher levels of the novel proinflammatory index MMP-2 in cow plasma and initial colostrum associated with SF68 infusion might imply subclinical systemic and mammary gland inflammation.

Lower casein composition in the initial colostrum might reveal suboptimal mammary synthetic capacity at partum, and, therefore, warrants more investigations.
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Thanks for your attention!

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