



ReaShure®-XC
Precision Release Choline

Choline's Impact on Calf Health & Growth Multi-Study Research Summary

Trial 1: Effects of supplementation with ruminally protected choline on performance of multiparous Holstein cows did not depend upon prepartum intake of calories

Zenobi, M.G., R. Gardinal, J. Zuniga, A. Dias, C. Nelson, J. Driver, B. Barton, J.E. Santos, and C. Staples. 2018a. J. Dairy Sci. 101:1088-1110.

Trial 2: Effects of maternal choline supplementation on performance and immunity of progeny from birth to weaning

Zenobi, M.G., J. M. Bollatti, A. M. Lopez, B. A. Barton, C. L. Hixson, F. P. Maunsell, W. W. Thatcher, K. Miller-Cushon, J. E. P. Santos, C. R. Staples, and C. D. Nelson. 2022. J. Dairy Sci. 105:9896-9916.

Trial 3: Increasing the prepartum dose of rumen-protected choline: Effects of maternal choline supplementation on growth, feed efficiency, and metabolism in Holstein and Holstein x Angus calves

Holdorf, H.T., W. E. Brown, G. J. Combs, S. J. Henisz, S. J. Kendall, M. J. Caputo, K. E. Ruh, and H. M. White. 2023. J. Dairy Sci. 106:6005-6027.

Balchem manufactures two versions of their rumen-protected choline product. ReaShure and a more concentrated form called ReaShure-XC. Both are designed to provide approximately 13 g/d choline ion when fed at the recommended feeding levels (ReaShure = 60g/h/d, ReaShure-XC = 30 g/h/d). One or both of these products were used in the following trials.

Choline's Role in Calf Health & Performance

Epigenetics is the study of how the *in utero* environment can modify gene expression without changing the genes themselves. DNA methylation is the most studied mechanism of epigenetics and involves adding a methyl group to a DNA molecule. DNA methylation regulates gene expression, effectively turning some genes on and off. One requisite for DNA methylation is the availability of molecules named methyl group donors, such as choline. Choline is an efficient methyl donor with three methyl groups in comparison to most others that offer only one.

Epigenetic changes experienced during gestation that modify the phenotype of the calf after birth are called fetal programming. The increased demand for choline during pregnancy and lactation represents a threat to the developing fetus if a sufficient supply of methyl group donors are unavailable to the dam, negatively affecting the epigenetic gene expression in the calf. There is significant evidence that choline deficiency during gestation can negatively affect placental development, fetal growth, cognitive function, and the offspring's immune system (Jiang et al., 2014).

The choline requirement for the pregnant dairy cow remains unknown, although there is vast evidence that supplementation is required for essentially every cow. Several trials have been conducted to further establish the impact of choline supplementation to pregnant, nonlactating dairy cows on calf performance. The general objective of the three studies summarized below was to investigate the effect of supplementing late gestation cows with ReaShure® Precision Release Choline (Balchem Corp., Montvale, NJ) on the growth and health of the resultant calves.

Methodologies

Trial 1. Zenobi et al. (2018a) Pregnant, nonlactating, multiparous Holstein cows were assigned randomly to 1 of 4 treatments in a 2 × 2 factorial arrangement. One factor was either 0 or 60 grams (12,9 g/d choline ion) of ReaShure per cow per day from 21 d before expected calving through 21 d postpartum. The second factor was prepartum diets formulated to deliver excess energy or enough energy for maintenance in relation to requirements. Retrospective data were analyzed for the *in utero* effect of choline on offspring performance. Female calves were weighed at birth, weaning, and 350 d of age and average daily gain was calculated. Note that all diets were balanced for methionine. Feeding and management of calves were the same across all treatments.

Trial 2. Zenobi et al. (2022) Pregnant, nonlactating, multiparous Holstein cows and their offspring (59 heifers and 52 bulls) were enrolled. Calves were exposed to choline biomolecules *in utero* through dietary supplementation of 0 or 60 g/d (12,9 g/d choline ion) of ReaShure to their dams the last 21 d of gestation and through consumption of colostrum (3.8 L within 2 h of birth) from dams supplemented with 0 or 60 g/d (12,9 g/d choline ion) of ReaShure in a 2 × 2 factorial arrangement of treatments. The two main treatment effects were prenatal supplementation with either 0 or 60 g/d (12,9 g/d choline ion) of ReaShure and the source of colostrum from dams fed either 0 or 60 g/d (12,9 g/d choline ion) of ReaShure during late gestation. Note that all diets were balanced for methionine. Growth, intakes, and immunity of females were evaluated up to 56 d of age. Blood cell types and rectal temperature were measured up to 21 d of age.

Trial 3. Holdorf et al. (2023) Pregnant, nonlactating, multiparous Holstein cows pregnant with female Holstein calves (n = 49) or Holstein x Angus calves (male, n = 18; female, n = 30) were randomly assigned to: 1) Control (0 g/d choline ion), 2) 15 g/d choline ion from ReaShure (pre- and postpartum), 3) 15 g/d choline ion from ReaShure-XC (pre- and postpartum) and 4) 22 g/d of choline ion from ReaShure-XC prepartum followed

Figure 1 Fever incidence during the first 21 days of life

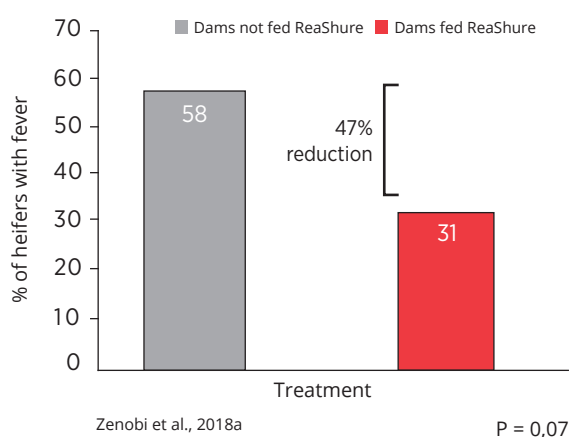
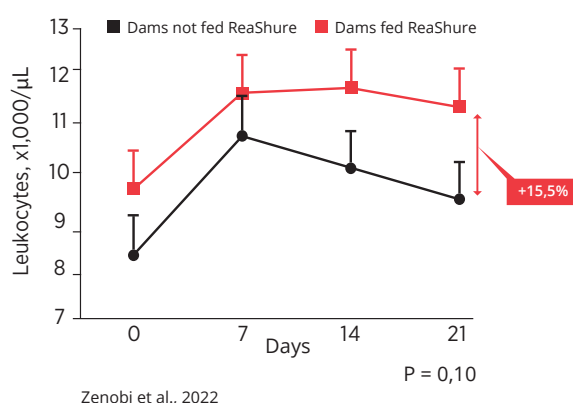


Figure 2 Heifers born from ReaShure-supplemented dams had increased concentrations of white blood cells (*In Utero* Effect)



by 15 g/d postpartum (21 d). Calves were fed colostrum post-calving from dams within the same treatments. Holstein calves were fed an accelerated milk replacer program, whereas the Holstein x Angus calves were fed a traditional milk replacer program. All calves were offered free choice access to calf starter. Jugular vein blood samples were collected, and body weight was measured weekly up to 56 d of age.

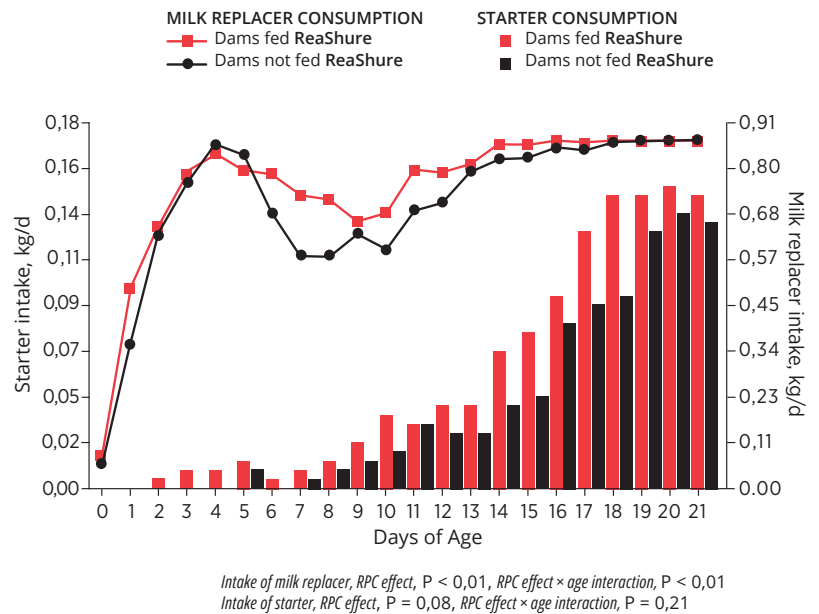
Results

Zenobi et al. (2018a) Calves exposed *in utero* to a richer choline environment during the last 3 weeks of gestation tended ($P = 0,07$) to be lighter at birth (40,4 kg vs. 38,7 kg) but weighed more ($P = 0,05$) by 350 d of age (335,5 kg vs. 322,5 kg), which tended ($P = 0,06$) to result in a greater ADG from birth to 350 d of age (0,81 kg/d vs. 0,85 kg/d) in heifers born from cows fed ReaShure.

Zenobi et al. (2022) Calves fed colostrum from ReaShure-supplemented dams had a 19.1% increase in apparent efficiency of IgG absorption compared with calves fed colostrum from control dams ($P = 0,01$; 27,4 vs. 23,0%). Incidence of fever in the first 21 d of age tended ($P = 0,07$; Figure 1) to be less in females born from ReaShure-supplemented dams compared with females born from control dams (31 vs. 58%). Neonatal females from dams fed ReaShure had increased ($P < 0,10$) hematocrit and concentrations of red blood cells, leukocytes, neutrophils, and lymphocytes in blood compared with neonatal females born from control dams. Compared with neonatal control females, neonatal females from dams fed ReaShure had greater (Figure 3) intake of milk replacer (704 g/d vs. 748 g/d) and starter (40,4 g/d vs. 53,6 g/d) during the first 21 d of age. From birth to 28 d of age, neonatal calves from dams fed ReaShure tended to have greater ADG as compared to neonatal calves in the control group ($P = 0,06$; 0,76 kg vs. 0,64 kg/d, respectively). Summing together females and males ($n = 111$), mortality rate during the first 24 days was reduced when calves were exposed to choline molecules before calving and fed colostrum harvested from dams supplemented with ReaShure. The mortality rate was the highest for calves born from the control dams and receiving colostrum from the same group (30%), intermediate for calves exposed to choline biomolecules either *in utero* (18%) or through colostrum (16%), and lowest (0%) from those calves exposed to choline both *in utero* and through colostrum.

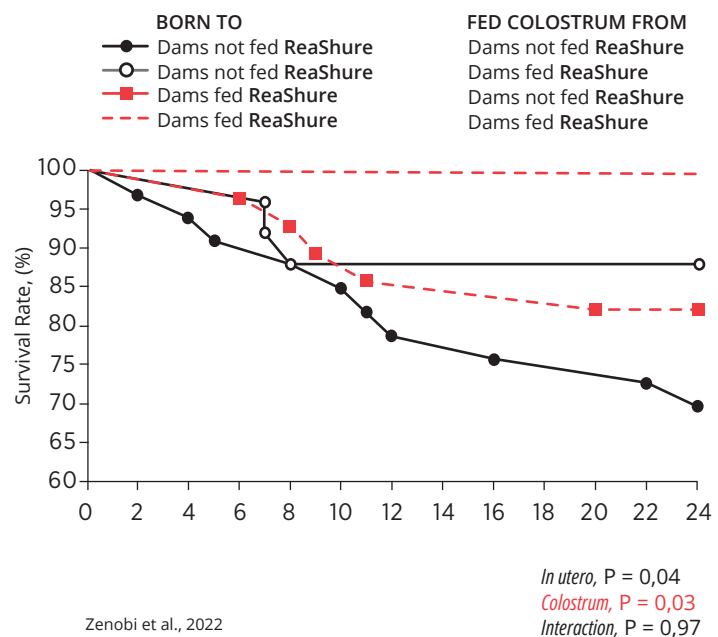
Holdorf et al. (2023) In the Holstein heifer calves, increasing ReaShure-XC dose linearly increased ($P \leq 0,03$) average daily gain and

Figure 3 Late gestation exposure to choline biomolecules increased DMI of milk replacer and starter during the first 21 d of age in heifers



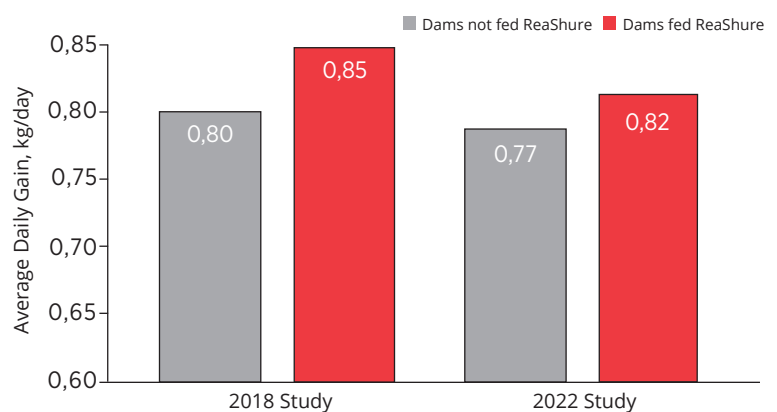
Zenobi et al., 2022

Figure 4 Survival curves by treatment



Zenobi et al., 2022

Figure 5 Summary of average daily gains for Holstein heifers through 350 days from two independent studies



Zenobi et al., 2018a
 Zenobi et al., 2022 (personal communication)

$P = 0,06$

feed efficiency during the first 14 days of life. All products/treatments (ReaShure and ReaShure-XC) increased ($P \leq 0,03$) blood glucose in Holstein heifer calves. At seven days of age, blood lipopolysaccharide binding protein (LBP) was decreased ($P < 0,01$) by feeding any RPC treatment in Holstein heifer calves. In male Holstein x Angus calves, the high dose of ReaShure-XC increased average daily gain after two weeks of life ($P = 0,02$) and also reduced ($P = 0,03$) blood urea nitrogen across the entire experimental period. However, regardless of RPC source, increasing maternal choline ion intake linearly increased ($P < 0,01$) average daily gain beyond two weeks of age for male Holstein x Angus calves, and quadratically increased ($P \leq 0,02$) feed efficiency in both male and female Holstein x Angus calves. Additionally, all choline treatments increased ($P < 0,01$) the proportion of methylated whole blood DNA in male, but not female, Holstein x Angus calves.

Discussion

It is intriguing to elucidate the mechanisms by which prenatal choline supplementation exerted these positive results on the offspring. One possibility is that heifers born from dams supplemented with ReaShure experienced optimized development and maturation of different organs, such as the gastrointestinal tract, the lungs, or even the immune system, as an indirect effect of *in utero* choline exposure. Alternatively, choline may directly act on the developing calf by introducing epigenome modifications. Deficiency of methyl group donors has been induced experimentally pre- and postnatal in many animal species, with consistent findings of a reduced number of red and white blood cells (Hoffbrand, 1978; Kumar and Axelrod, 1978). The lower concentrations of white and red blood cells support the hypothesis that a broad segment of the immune system is affected *in utero* by an insufficient supply of choline. This negative effect is not corrected solely by postnatal supplementation of choline via colostrum, leading to long-term negative consequences. Also, maternal choline supplementation has been shown to influence placental nutrient transporter abundance and nutrient metabolism in late gestation, thereby influencing nutrient supply to the fetus (Kwan et al., 2017). Taken together,

these pieces of evidence support the concept that choline plays a key role in prenatal programming of the offspring. Interestingly enough, the new results seem to confirm this as a phenomenon across all species.

Summary

Choline is a crucial molecule whose availability determines the outcome of epigenetic modulation of gene expression. Optimizing fetal programming events with ReaShure supplementation during the last three weeks of gestation can lead to improved long-term growth and health of the offspring. Thus, supplementing dairy cows with ReaShure during the transition period can be a sound investment for the future of your dairy.*

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