GOLDILOCKS MEETS CHOLINE, TOO HOT? TOO COLD? JUST RIGHT?

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Background. Choline has been identified as a required nutrient for many species including humans, chicks, and pigs. Choline is found in low concentrations in most feeds, ranging from 0.04% in corn silage and alfalfa hay to 0.3% in protein sources such as soybean meal and cottonseed meal (DM basis). Its low concentrations in feeds are indicative of the low amounts required by livestock (e.g. 3 g/day for a lactating sow). Although the bovine requirement for choline has yet to be established, the supplementation of choline to dairy cows in transition usually improves milk production and often aids in the reduction of fat in the liver. However, in order for choline to be absorbed in the small intestine of ruminants, the choline must have some protection from degradation by ruminal microbes which degrade dietary choline to methane and acetic acid. Several ruminally-protected choline (**RPC**) products are being marketed commercially to the dairy industry across the world. ReaShure (Balchem Corp., New Hampton, NY) is such a product containing approximately 25% choline chloride. In 16 experiments published since 2003, dairy cows supplemented with an RPC product starting in late gestation (~ 3 weeks prepartum) produced an average of 4.4 lb/day more milk or fat-corrected milk compared with cows not supplemented with RPC. Ten of the 16 studies reported a significant increase in milk due to RPC supplementation. This increase in milk due to choline supplementation often has been explained through choline's role to improve lipid metabolism by the liver. The liver of the modern dairy cow accumulates fat (triacylglycerol, **TAG**) in the early weeks after calving because of the massive mobilization of adipose tissue for energy use during the extensive period of negative energy balance (**NEB**). The efficiency of the excessively fat liver to manufacture glucose for milk synthesis is compromised resulting in reduced milk yield. The liver can export some of the fat with the aid of choline. Removing choline from the diet is a way that researchers often create fatty liver in nonruminant species. When animals are pregnant or lactating, concentrations of choline in the liver decrease dramatically (Zeisel, 2000).

Proper body condition at calving is important for optimal milk yield. Thin cows lack the energy reserves to support needed energy for milk synthesis during the inevitable negative energy state whereas fat cows are often poor eaters and experience greater states of negative energy postpartum resulting in even greater fatty liver, reduced milk yield, and poor reproductive performance. Excessive fat reserves are oftentimes hidden from view; fat that is stored in the abdomen around the intestines and the kidneys is not considered when one is condition-scoring cows. Excessive energy intake during the dry period can build up this abdominal (visceral) fat without changing the overall body condition of the cow (Drackley et al., 2014). Nonlactating and nonpregnant Holstein cows were fed a lower energy diet (0.61 Mcal/lb) of 41% wheat straw and 28% corn silage or a higher energy diet (0.735 Mcal/lb) of 0% wheat straw and 50% corn silage (DM basis) in ad libitum amounts. After 8 weeks on the 2 diets, body condition was the same, 3.47 vs. 3.52, respectively. Upon slaughter, it was discovered that the cows fed the lower energy diet had 56 fewer pounds of abdominal fat (70 vs. 126 lb). Feeding

prepartum diets that better match the energy requirement for maintenance and pregnancy is the "just right" (a.k.a. the "Goldilocks") approach. Eating the porridge at the right temperature, sitting in the chair that is the right height, and sleeping in the bed that is the most comfortable is not extreme to either side. Likewise, underfeeding or overfeeding energy to pregnant cows during the whole dry period ends up damaging cow performance postpartum. In the last 2 decades of research in this area of transition cow feeding, formulating well-balanced diets containing substantial proportions of low quality forages such as wheat straw has resulted in better health of the postpartum cow (Drackley, 2016). Too often, cows are overfed during the dry period because a dairy will feed the TMR refused by the lactating cows. This approach may not appear to be harmful because body condition appears "normal" but research indicates that dangers lurk like the fury of a moma bear.

Experimental hypothesis and approach. Because overfeeding energy during the dry period often leads to fatty liver and because choline plays a key role in improving the liver's management of fat, it was hypothesized that choline supplementation would most benefit those dairy cows overfed energy during the dry period.

Ninety-six pregnant, nonlactating multiparous Holstein cows (University of Florida) were assigned to 1 of 4 dietary treatments on the day of 'dry off' (~7 weeks prior to expected calving date). Dietary treatments were arranged in a 2 × 2 factorial. One factor was RPC (ReaShure, Balchem Corp., New Hampton, NY) top-dressed once daily at 0 or 60 g/day per cow from 21 days prior to expected calving date through 21 days postpartum. The second factor was diets of 0.74 (excess energy) or 0.64 (maintenance energy) Mcal of NE_L/lb of dietary DM fed in ad libitum amounts for the whole dry period. Therefore, the 4 treatments were maintenance energy intake without RPC (**MNE**) or with RPC (**MNE+C**) and excess energy intake without RPC (**EXE**) or with RPC (**EXE+C**). Chopped wheat straw (< 2 inches), corn silage, and triticale silage were adjusted to formulate to the targeted energy density of the prepartum diets. Wet brew was added to the TMR (16.7% of dietary DM) to minimize sorting by the cows managed in a Calan gate system. At the time of enrollment, parity (1.9), 305-day mature equivalent milk production (26,701 lb), body condition score (3.55), or body weight (1622 lb) did not differ between the 4 groups of cows. After calving all cows were fed the same basal diet (0.76 Mcal NE_L per lb and 16.0% CP, DM basis) through 15 weeks postpartum when the trial ended. Diets were formulated to have methionine at 2.3% of metabolizable protein and a lysine-to-methionine ratio of 2.9 prepartum and 3.1 postpartum. Measurements taken included intake of feed, body weight and condition, yield and IgG content of colostrum, health disorders, milk production and composition, triacylglycerol content of liver via biopsy, uterine health assessments, selected metabolites and immune responses in blood, and pregnancy to timed artificial insemination.

Data were analyzed using the MIXED procedure of SAS version 9.4 (SAS/STAT, SAS Institute Inc., Cary, NC). The REPEATED statement was used for dependent variables measured over time. Models included the fixed effects of energy intake prepartum (excess vs. maintenance), RPC (with vs. without), interaction between energy intake prepartum and RPC, day or week of measurement, and all 2- and 3-way interactions. Cow was nested within treatment and was the

error term for testing the effects of treatment. Data were transformed to achieve normality if needed before analyses. Binary data were analyzed by logistic regression using the GLIMMIX procedure of SAS. Time to event such as interval to pregnancy by 210 DIM was analyzed with Cox's proportional hazard regression model using the PHREG procedure of SAS. Statistical significance was considered at $P \leq 0.05$ and tendency was considered at $0.05 < P \leq 0.10$.

Experimental results and discussion. For nearly every dependent variable, the influence of each main treatment effect was independent. That is, the effect of choline was the same if the cow was fed the lower energy diet or the greater energy diet prepartum. Likewise, the effect of prepartum energy intake was the same regardless of whether the cow was supplemented with choline. Therefore, the main effects of prepartum energy intake and choline will be presented separately.

Effects of prepartum energy intake.
Prepartum responses. Body condition score from dry-off to calving was unchanged. Mean DM intake during the last 15 days of gestation (mean of 23.7 lb/day) did not differ due to energy density of the diets. However intake of energy did differ between the 2 groups as planned. Two weeks prior to calving, cows fed the EXE diet were consuming energy at 140% of their requirement for maintenance and pregnancy whereas cows fed the MNE diet were eating at 110% of their requirement (NRC, 2001). The pattern of NE_L intake over the last 2 weeks of gestation also differed [$P < 0.01$, energy diet by day interaction (Figure 1)]. As reported by many others, intake of energy decreased as parturition approached. However, the intake of NE_L by cows fed the EXE diet decreased at twice the rate compared to that by cows fed the MNE diet, dropping the equivalent to 0.6 vs. 0.3 lb per day or a total of 9 (34%) and 4.5 lb (20%), respectively. As a result of the greater NE_L intake prepartum, mean concentration of nonesterified fatty acids (**NEFA**) tended to be lower (252 vs. 295 µEq/mL, $P < 0.10$) and that of glucose was greater (66.4 vs. 63.5 mg/100 mL, $P < 0.05$) in plasma of cows fed the EXE compared with the MNE diet although values were within the normal range for well-managed prepartum dairy cows.

Postpartum responses. Cows fed the EXE diet prepartum consumed 2.7 lb less feed DM ($P < 0.01$) during the 15-week postpartum period compared with cows fed the MNE diet (50.4 vs. 53.1 lb/day, respectively). This response is rarely significant although numerically lower postpartum DM intake by cows overfed energy prepartum has been reported previously (Holcomb et al., 2001; Dann et al., 2006; Zhang et al., 2015). However, mean production of milk over the first 15 weeks postpartum was not different (91.9 vs. 95.1 lb/day of uncorrected milk yield [$P = 0.25$] and 93.9 vs. 96.2 lb/day of energy-corrected milk yield [$P = 0.38$] for cows consuming EXE and MNE diets, respectively). Concentration of fat (3.88 vs. 3.78%) and true protein (2.95 vs. 2.97%) in milk were not affected by prepartum energy intake. The gross efficiency of converting feed DM into ECM almost reached a significant tendency favoring cows fed the EXE diet (1.90 vs. 1.84 lb of milk per lb of feed intake, $P = 0.11$). This improved gross efficiency of milk from feed came at the cost of body reserves. After body weight of both groups of cows hit a low after 4 weeks of lactation, cows from the EXE treatment simply maintained their body weight the rest of the way whereas cows from the MNE treatment

started gaining weight until they put on ~70 lb at 15 weeks postpartum. This greater reliance on body reserves for the milk that was produced by cows fed EXE diets prepartum is reflected in greater mean concentrations of circulating beta-hydroxybutyric acid (**BHBA**; 0.52 vs. 0.43 mmol/L, $P < 0.05$) and NEFA (502 vs. 453 µEq/mL, $P < 0.10$). As a result of greater fat circulating in the blood, the liver of cows fed EXE diets accumulated more TAG fat at 7 (11.1 vs. 8.7% of DM) and 21 (10.1 vs. 7.6% of DM) days in milk compared with cows fed MNE diets prepartum.

Fatty liver is often associated with ketosis and reduced reproductive performance. Incidence of health disorders were recorded although the study lacked sufficient numbers of cows to adequately test the effect of prepartum energy intake. Incidence of diseases/disorders that reached a probability of significance of ≤ 0.20 due to feeding EXE diets included ketosis (16.9 vs. 10.2%) and uterine infection at 40 days in milk (15.2 vs. 7.1%). However excess energy intake prepartum did not influence ovarian cyclicity postpartum either at 26 (45.1 vs. 60.6%) or at 40 (78.7 vs. 82.5%) days in milk compared to cows fed MNE diets as determined by the presence of a corpus luteum detected using ultrasonography. Pregnancy at first AI was 32% for both treatment groups.

Effects of choline supplementation.
Prepartum responses. Although pregnant cows began RPC supplementation at 21 days prior to expected calving date, cows consumed supplemental RPC for only the last 17 days of gestation on average because they calved earlier than expected. Supplementing RPC did not change mean DM intake during the last 15 days (23.1 vs. 24.2 lb DM/day for –RPC and +RPC-fed cows, respectively). Body condition score of cows averaged 3.51 and did not differ due to RPC feeding. Blood concentrations of NEFA and BHBA also were unaffected by RPC supplementation.

Postpartum responses. Yield of colostrum was not affected by RPC supplementation (18.8 vs. 21.8 lb) but colostrum from cows fed RPC had a greater concentration of immunoglobulin G (**IgG**; 78 vs. 57 g of IgG/L). The source of colostrum that was fed to the calves born from the cows on this study was not controlled. Nevertheless, the growth of the calves over the following 12 months of life was affected by being exposed to RPC in utero. Calves born to dams supplemented with RPC tended to be 4.6 lb lighter at birth (84.5 vs. 89.2 lb, $P < 0.10$) but were 31 lb heavier at 12 months of age (739 vs. 7089 lb, $P < 0.05$) thus growing at 0.1 lb/day faster compared to calves born from unsupplemented dams (1.97 vs. 1.87 lb/day). Apart from the colostrum, all calves were managed the same during this time period. Choline has been helpful in the diet of nonruminant animals during pregnancy to improve offspring performance (Newberme et al., 1970; Zeisel, 2006). Whether this may be true for dairy calves should be investigated in the future.

As occurred in the prepartum period, intake of feed DM postpartum was not affected by RPC supplementation (52.3 vs. 51.1 lb/day) although the 1.2 lb/day numerical increase due to RPC supplementation is the same increase as that reported by Grummer (2012) using a meta-analysis of RPC-feeding studies with lactating dairy cows. Cows supplemented with RPC tended ($P < 0.10$) to produce more milk during the first 15 weeks of lactation (95.9 vs. 91.0 lb/day). This

tendency for increased milk yield detected during the first 15 weeks continued for 40 weeks of lactation (81.7 vs. 77.1 lb/day, $P < 0.10$; Figure 2). Holstein cows produced nearly 5 more pounds per day of milk for 40 weeks of lactation when supplemented with 15 g of choline chloride for approximately 5.5 weeks over the transition period. This milk increase is similar to that reported by Elek et al. (2008), Janovick et al. (2006), and Lima et al. (2012) and to that reported in the meta-analysis by Grummer (2012). Although concentration of fat (3.82 vs. 3.84%) and true protein (2.95 vs. 2.97%) in milk were not affected by RPC, the yield of both fat and protein tended to be greater by cows fed RPC due to their tendency for greater milk yield. Greater milk yield without a significant increase in feed intake resulted in a greater mean NEB of cows fed RPC over the 15 weeks (-1.18 vs. -0.53 Mcal/day). The pattern of NEB over the 15 weeks postpartum also differed between groups. Cows fed RPC were experiencing a more NEB in weeks 2 (-11.4 vs. -8.9 Mcal/day) and 3 (-8.7 vs. -6.6 Mcal/day) postpartum. No difference in energy balance occurred between groups after cows moved past week 6 (RPC by week interaction, $P < 0.10$). Despite a greater NEB, loss of body weight from calving to week 4 postpartum was not different (101 vs. 83 lb). In addition, mean concentrations of NEFA and BHBA in blood were not affected.

Treatment for ketosis was the only disease/disorder that reached a probability of significance of ≤ 0.20 due to feeding RPC (18 vs. 9% for +RPC vs. –RPC, respectively). Diagnosis of ketosis was based upon ketostix classification of urine BHBA as 'moderate' (~40 mg/100 mL) or 'large' (80 mg/100 mL). In a field study using more cows (n = 369), primiparous and multiparous cows were fed 15 g/d of RPC from 25 days prepartum to 80 days postpartum (Lima et al., 2012). Yield of fat-corrected milk increased 4 lb/day (98.3 vs. 94.3 lb/day) due to RPC feeding. Cows fed RPC had less morbidity, especially less clinical ketosis (4.7 vs. 13.9% for primiparous cows and 3.5 vs. 9.8% for multiparous cows). Other measures that are indicators of cow health suggest a positive influence of RPC in the current study. Rectal temperature measured at 4, 7, and 12 days in milk decreased linearly from 101.8 to 101.2°F for RPC-supplemented cows whereas that for –RPC cows increased linearly from 101.6 to 101.9°F. A concentration of < 8.5 mg of total Ca/100 mL of blood plasma was used as a definition of subclinical milk fever in blood samples collected at 0, 1, 3, and 7 days in milk (Chapinal et al., 2012; Martinez et al., 2012). Cows fed RPC had greater mean concentrations of Ca across measurement days (8.72 vs. 8.46 mg/100 mL) and the prevalence of subclinical milk fever (using any of the 4 days of measurement) was reduced ($P < 0.05$) from 52.1 to 31.6%.

The pattern and the mean concentration of TAG in liver over 7, 14, and 21 days in milk was not affected by RPC supplementation (8.2 vs. 7.4% TAG DM basis for +RPC and –RPC, respectively). This lack of effect of RPC on liver TAG is in agreement with Zahra et al. (2006) and Piepenbrink and Overton (2003). However several studies have reported reduced TAG concentrations in the liver of lactating dairy cows in the early postpartum period including Elek et al. (2013), Santos and Lima (2009), and Zom et al. (2011). The TAG values in the current FL study were quite low and may have been less susceptible to TAG reduction by RPC.

The greater NEB of cows fed RPC did not influence the proportion of cows cycling at 26 and 40 days in milk based upon a detectable corpus luteum. However pregnancy at first insemination

tended to favor cows fed RPC (41.3 vs. 23.6%, *P* < 0.10) although the proportion of cows pregnant by 40 weeks postpartum did not differ (69.8 vs. 62.5%). In a study conducted at a commercial dairy in California using both primiparous and multiparous cows (Lima et al., 2012), pregnancy rate after the first and second insemination was numerically but not significantly better due to feeding RPC from 25 days pre-calving to 80 days post-calving (59.8 vs. 52.7%).

Summary. Supplementing ruminally protected choline chloride at 15 g/day from approximately 17 days prepartum to 21 days postpartum resulted in greater (P < 0.10) yield of milk (4.9 lb/day) and milk components through 40 weeks of lactation, greater NEB at 2 and 3 weeks postpartum without changing TAG in liver, greater concentration and yield of IgG in colostrum, greater pregnancy at first insemination, and better daily gains in body weight by calves from those dams regardless of the amount of energy consumed during the entire dry period. Compared with feeding to maintenance, overfeeding energy by 40% during the dry period resulted in a greater decrease in DM intake as day of calving approached, lower intake of DM (2.5 lb/day) with no change in milk yield, greater concentrations of fat in blood and liver, and greater delay in gaining body weight postpartum.

References

Chapinal, N., M. E. Carson, S. J. LeBlanc, K. E. Leslie, S. Godden, M. Capel, J. E. P. Santos, M. W. Overton, and T. F. Duffield. 2012. The association of serum metabolites in the transition period with milk production and early-lactation reproductive performance. J. Dairy Sci. 95:1301-1309.

Dann, H.M., N.B. Litherland, J.P. Underwood, M. Bionaz, A. D'Angelo, J.W. McFadden, and J.K. Drackley. 2006. Diets during far-off and close-up dry periods affect periparturient metabolism and lactation in multiparous cows. J. Dairy Sci. 89:3563-3577.

Drackley, J.K. 2016. "The "Goldilocks diet" 10 years on…is it still "just right"? in Proc. SW Nutrition Conf. Tempe, AZ. pp. 129-141.

Drackley, J.K., R.L. Wallace, D. Graugnard, J. Vasquez, B.F. Richards, and J.J. Loor. 2014. Visceral adipose tissue mass in nonlactating dairy cows fed diets differing in energy density. J. Dairy Sci. 97:3420-3430.

Elek, P., T. Gaal, and F. Husveth. 2013. Influence of rumen-protected choline on liver composition and blood variables indicating energy balance in periparturient dairy cows. Acta. Veterinaria Hungarica 61:59-70.

Elek, P., J.R. Newbold, T. Gaal, L. Wagner, and F. Husveth. 2008. Effects of rumen-protected choline supplementation on milk production and choline supply of periparturient dairy cows. Animal 2:1595-1601.

Grummer, R.R., 2012. Choline: A limiting nutrient for transition dairy cows. Pages 22-27 *in Proc. Cornell Nutr. Conf. Cornell University, Syracuse, NY.*

Holcomb, C.S., H.H. Van Horn, H.H. Head, M.B. Hall, and C.J. Wilcox. 2001. Effects of prepartum dry matter intake and forage percentage on postpartum performance of lactating dairy cows. J. Dairy Sci. 84:2051-2058.

Janovick Guretzky, N.A., D.B. Carlson, J.E. Garrett, and J.K. Drackley. 2006. Lipid metabolite profiles and milk production for Holstein and Jersey cows fed rumen-protected choline during the periparturient period. J. Dairy Sci. 89:188-200.

Lima, F.S., M.F. Sa Filho, L.F. Greco, and J.E.P. Santos. 2012. Effects of feeding rumen-protected choline on incidence of diseases and reproduction in dairy cows. Vet. J. 193:140-146.

Martinez, N., F.S. Lima, R.S. Bisinotto, L.F. Greco, E.S. Ribeiro, F. Maunsell, K.N. Galvão, C.A. Risco, and J.E.P. Santos. 2012. Evaluation of peripartal calcium status, energetic profile, and neutrophil function in dairy cows at low or high risk of developing uterine disease. J. Dairy Sci. 95:7158-7172.

Newberne, P.M., R.B. Wilson, and G. Williams. 1970. Effects of severe and marginal maternal lipotrope deficiency on response of postnatal rats to infection. Brit. J. Exp. Path. 51:229.

Piepenbrink, M.S., and T.R. Overton. 2003. Liver metabolism and production of cows fed increasing amounts of rumen-protected choline during the periparturient period. J. Dairy Sci. 86:1722-1733.

NRC, 2001. Nutrient requirements of dairy cattle. 7th rev. ed. Natl. Acad. Press, Washington, DC.

Santos, J.E.P., and F.S. Lima. 2009. Feeding rumen-protected choline to transition cows. Pages 149-159 in Proc. Florida Ruminant Nutrition Symposium. Univ. FL, Gainesville.

Zang, Q, H. Su, F. Wang, Z. Cao, and S. Li. 2015. Effects of energy density in close-up diets and postpartum supplementation of extruded full-fat soybean oil on lactation performance and metabolic and hormonal status of dairy cows. J. Dairy Sci. 98:7115-7130.

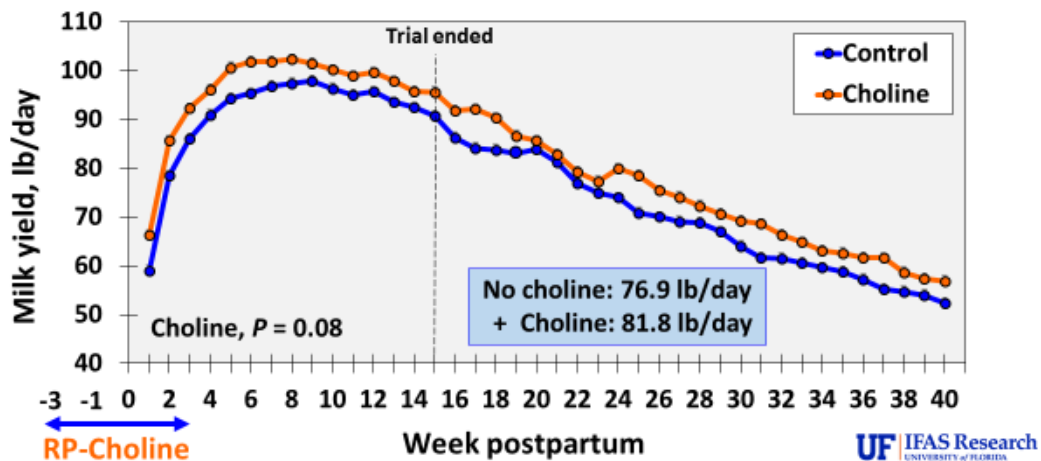Zeisel, S.H. 2000. Choline: An essential nutrient for humans. Nutrition 16:669-671.

Zeisel, S. H. 2006. Choline: Critical role during fetal development and dietary requirements in adults. Annual Rev. Nutr. 26:229 -250. http://doi.org/10.1146/annurev.nutr.26.061505.111156

Figure 1. Effect of Prepartum Diet on Energy Intake
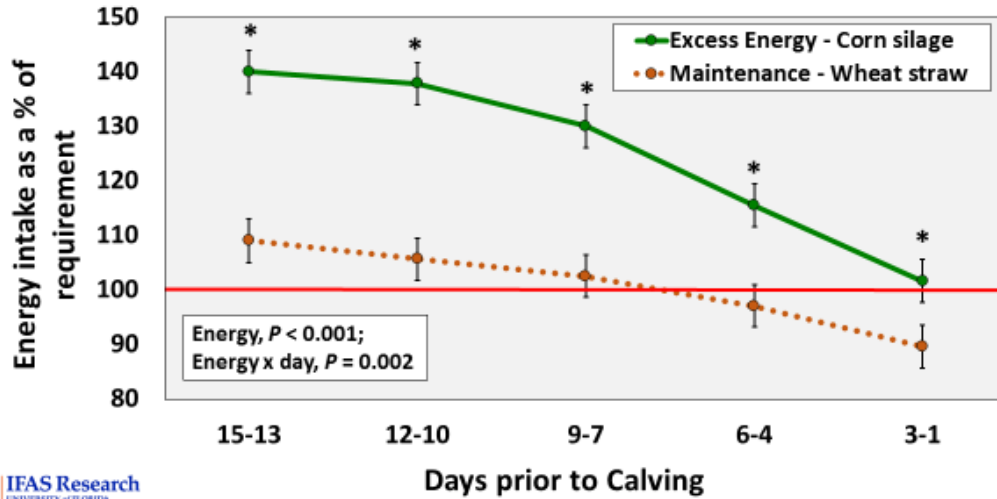
Figure 2. Positive Benefits of Ruminally-Protected Choline Continued After Supplementation Ceased – 40 Weeks