INTRODUCTION

There are few scientific meetings and symposia that do not explore the impact of nutrition on reproduction. Additionally, there have been many manuscripts published in the area. Most studies have focused on the effects of a single nutrient fed in various amounts relative to recommended requirements on some reproductive parameter. Certainly, the subjects of energy nutrition and to a lesser extent protein/amino acid and fatty acid nutrition have been given attention in the literature. Other nutrients, co-factors, and co-enzymes have also been given less, but some attention (minerals, antioxidants, choline, niacin, etc.). Fewer publications exist on the effect of specific nutrients during the prepartum transition period on reproductive outcomes and some of these will be reviewed in this manuscript.

Common sense tells us that nutrition impacts reproduction, regardless of the point that the cow is at in her life cycle. Maybe it is time we shifted gears and, rather than treating reproduction as a series of stages that are influenced by specific nutritional factors, we begin to think of reproduction as a continuous process in which nutrition plays a fundamental role. In this regard there are certain factors that we should agree upon. For the purpose of this manuscript I will assume that:

- Nutrition/nutrient balance impacts reproductive performance at all stages of the life cycle;
- No single required nutrient impacts nutrition more or less than others;
- In terms of nutrition, each reproductive process along the path from insemination to calving is impacted by a specific set of nutrients; and
- Failure of any reproductive process should be considered a metabolic disease that is impacted by other health and immune issues as well as nutrition.

For the remainder of this manuscript, we will examine a few nutrient and nutritional interactions related to reproductive processes that have not been well summarized; point to a few excellent reviews related to other nutrients and their relationships to reproductive processes, and conclude with a discussion on state of the art of modeling reproductive processes in an attempt to find the weak links in any particular situation.

SPECIFIC NUTRIENT INTERACTION WITH REPRODUCTION

Energy, Energy Balance and Body Condition Score

There have been some excellent recent reviews of this area of nutrition by Santos (2011), Garnsworthy (2008), and Butler (2003). To summarize, excessive body condition prepartum lead to reduced dry matter intake (DMI) postpartum as well as calving difficulties. Reduced DMI postpartum leads to severe negative energy balance affecting production, body weight loss, and reproduction via interference with ideal hormonal signaling pathways and secretion. This not only interferes with the resumption of ovarian activity postpartum, but also results in a reduced quality and viability of oocytes that are ovulated and smaller corpora lutea that theoretically will secrete less progesterone.

There is literature on prepartum undernutrition of energy; however, none have looked specifically at reproductive processes.

Protein and Amino Acid Nutrition

Although there is little information on underfeeding protein, specifically in the form of metabolizable protein (MP), in the early postpartum period on reproduction; common sense would lead us to believe that this practice would certainly lead to underperformance in production, reproduction, and immune responses. A larger practical question is “What are the effects of overfeeding protein relative to productive requirements during the postpartum period relative to reproductive performance?”

Lean et al. shared their submitted manuscript with me for the purposes of writing this manuscript. The results were summarized at the 2011 FASS meetings (Lean et al., 2011). In their meta-analysis...
and regression, they found that increased crude protein content or degradability of diets reduced fertility of dairy cattle and was consistent with the previous conclusions of Butler (1998), Ferguson and Chalupa (1989) and Westwood et al. (1998). The estimated reduction in risk of conception was relatively modest (~10%), but reflected findings within the range of differences in protein content or degradability examined in the dietary comparisons of the studies included. These findings support the mechanisms of reproductive failure that were reviewed by Butler (1998) and associated with increased concentrations of nitrogen or ammonia in the blood or uterus; but do not preclude other suggested mechanisms of effect including effects on nutrient balance, specifically energy, protein, mineral, or vitamin balance.

The lack of significant association between blood urea nitrogen (BUN) and risk of conception does not preclude a role of increased concentrations of urea in blood affecting fertility, but suggest that there is either difficulty in measurement or a limited role for the increase per se. Although some cited literature makes the association between BUN, with milk urea nitrogen (MUN) serving as its proxy, and reduced risk of conception; there is a fair amount of inconsistency in the data as pointed out in the study by Lean et al. (2011). Guo et al. (2004) showed that while MUN is associated with reduced reproductive performance within herds, there is a lack of relationship between herds making it difficult to cite a specific value for MUN that would be considered safe. The reason for this phenomenon is likely because it is more important as to why MUN is high or low rather than the fact that it is high or low. Certainly MUN can be increased by high intake of degradable protein, MP in excess of requirement, negative energy balance (forcing deamination of amino acids and protein catabolism for carbon skeleton use), and amino acid imbalances (again forcing deamination and reducing efficiency of nitrogen use).

The question arises, why do we see productive responses and apparent yet equivocal reproductive successes when we intentionally overfeed protein relative to requirements of early postpartum cows in the field? The contention of this discussion is that cows respond early postpartum to overfeeding protein because they are repleting protein stores lost in the prepartum transition period due to underfeeding MP and having amino acid imbalances. The competition between repleting protein stores with milk production, immune function, and reproduction causes the latter to suffer. In response we overfeed protein in early lactation, which may increase BUN/MUN and apply downward pressure on reproductive performance.

A good example of the above discussion is the work by Van Saun et al. (1993). This study was investigating the effect of prepartum undegradable intake protein (UIP) on postpartum performance and some reproductive parameters. The prepartum UIP was formulated to meet NRC requirements for the low UIP diet and exceed these for the high UIP diet. All cows received the same postpartum diet formulated to meet NRC requirements for crude protein. Upon recalculation of the diets used by entering them in to CNCPS v6.1, the prepartum MP intake for the low and high UIP diets were 900 and 1200g/d. Figure 1 shows the postpartum milk protein production parameters for the 2 groups of cows. The group fed low MP prepartum consistently produced lower percentages of milk protein. More importantly, this group produced less kg of milk protein between 14 and 35 days in milk (DIM). The shape of the line indicates that the cows fed low MP prepartum were unable to synthesize milk protein during this time, suggesting that postpartum MP intake was being used for other functions than milk production. Interestingly, although energy balance was not recorded, cows fed reduced MP prepartum lost more condition in the first 2 wk postpartum even though they were all fed the same postpartum diet. The only reproductive parameters recorded were services/conception (SPC) and days open (DO). Although not significant due to small cow numbers, the values for low and high MP for SPC were 2.1 vs. 1.2, respectively and for DO were 125 vs. 113, respectively.
Regardless of the type of prepartum transition diet we formulate (low or high fiber, reduced non-fiber carbohydrate (NFC) or not) we can significantly underfeed MP prepartum if we do not consider the state of the cow in the transition period and adjust whatever model we use to formulate diets for these cows. We do not normally adjust our ration formulation models for twinning, higher-than-normal calf birth weight, or the reduction in feed intake that occurs in the week before calving. These factors place enormous pressure on the requirement for MP and amino acids.

The suggestion is to formulate diets to achieve a MP intake of approximately 1100 to 1200 g/d for mature Holsteins and 1200 to 1300 g/d for primiparous Holstein heifers (to account for growth requirement). Following these recommendations may allow for feeding MP to postpartum cows closer to requirements without overfeeding and reducing the risk of MUN affecting reproduction while not sacrificing production performance.

Fats and Fatty Acid Nutrition: The Case for Reproduction and Immune Function

At a previous meeting of DCRC, Staples et al. (2008) presented data showing that adding fat to otherwise isocaloric diets improves conception rates. They go on to suggest that the unsaturated fatty acids might have more influence on this phenomenon than the saturated fatty acids and that the role might be more isolated to the essential fatty acids (EFA), n-6 linoleic acid and n-3 linoleic acid, DHA and EPA. The suggested mechanism by which the EFA might be exerting a positive effect on reproduction was suggested by Staples et al. (2008) to be via increasing the size of the ovarian follicle, production of higher quality embryos, reducing embryonic losses, and improving immune status. Data was presented supporting these hypotheses.

The physiology and nutritional biochemistry of the EFA are basically that the n-6 EFA is associated with synthesis of the prostaglandin (PG) 2 series and the inflammatory immune response system and the n-3 EFA are associated with the PG 3 series and the anti-inflammatory immune response system. Both EFA are needed in the synthesis of various reproduction hormones and other eicosanoids.

While there is a case for a need of n-3 EFA around the time of implantation to suppress the rejection of embryos and reduce embryonic loss, there is also a case for the n-6 EFA to heighten the inflammatory response for almost the entirety of the reproductive process. Therefore, it is likely that cows need both EFA all the time to allow for optimal functioning of the immune system and production of PG, hormones, and other eicosanoids. However, we do not have any indications as to the absolute requirements for either of the EFA at this point.

Further support for the concept of supplementing EFA exists in the literature. Jones et al. (2008) conducted a trial to compare reproductive outcomes in a commercial dairy herd fed Megalac® (calcium salts of long-chain fatty acids, Ca-LCFA, n = 1,312) or Megalac-R® (Ca-LCFA+EFA, n = 708; Church and Dwight Co. Inc., Princeton, NJ). Both products
are rumen-inert fat supplements of Ca-LCFA, although Megalac-R contains higher amounts of linoleic and linolenic acids. Beginning at 21 d prepartum, every cow received 0.11 kg/d of Ca-LCFA. All cows were randomly assigned to either product beginning at parturition and continuing until 150 d postpartum (0.16 kg/d). Ultrasonography was utilized to monitor ovarian status on a subset of cows < 30 DIM. Milk samples were collected for progesterone analysis and prostaglandins were prescribed by the herd veterinarian as a uterine therapy. Approximately 250 cows were subjected to an Ovsynch regimen to evaluate conception rates. Ultrasound results indicate that by 30 DIM, 49% of cows fed Ca-LCFA+EFA had ovulated compared with 27% of cows fed Ca-LCFA (P < 0.005).

Progestrone profiles from milk sample analysis for a subset of multiparous cows showed means of 2.0 and 2.8 estrous cycles per cow by 60 DIM for the Ca-LCFA and Ca-LCFA+EFA treated cows, respectively (P < 0.05). Within the first 60 DIM, 38.9% of cows fed Ca-LCFA and 29.0% of cows fed Ca-LCFA+EFA required prostaglandin treatment (P < 0.005). No difference was detected between treatments in terms of conception rates and services per conception. In summary, the higher concentrations of linoleic and linolenic acids in Ca-LCFA+EFA contributed to improved uterine health and ovulation rates early postpartum compared with Ca-LCFA.

In an unpublished research report Greco et al. (2010) describe a trial where periparturient cows were fed pre-and postpartum diets containing no added fat or added fat in the form of saturated fatty acids or Ca-LCFA containing EFA. Some of the clinical findings are in Table 1. Although statistical procedures could not be evaluated due to trial size (25 cows per treatment), numerically it appears that supplementation with EFA did affect immune responses via the apparent reduction in fevers, endometritis, and retained placentas while numerically increasing pregnancies at first AI

The link between uterine health and reproductive performance is one of common sense. The link between transition cow nutrition and immune function has been reviewed at a previous DCRC meeting (Goff et al., 2006). Basic biology of the immune system points to the role of PG and other eicosanoids in the inflammatory and anti-inflammatory responses. Goff et al. (2006) directly point to nutrient balance, hypocalcaemia, and other interacting metabolic issues as factors that can suppress immunity and increase the incidence of uterine problems.

Table 1. Clinical parameters for cows fed no added fat (Control) or added fat as saturated fatty acids (SFA) or calcium salts of long chain fatty acids with essential fatty acids (EFA) in the prepartum and postpartum rations (Greco et al., 2011 unpublished)

<table>
<thead>
<tr>
<th>Clinical Parameter</th>
<th>Treatment</th>
<th>Differences</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>SFA</td>
</tr>
<tr>
<td>Retained placenta</td>
<td>3.9</td>
<td>16</td>
</tr>
<tr>
<td>Fever, (&gt;39.5°C at 4, 7, and 12 DIM)</td>
<td>11.5</td>
<td>6.8</td>
</tr>
<tr>
<td>Clinical Endometritis</td>
<td>53.9</td>
<td>56</td>
</tr>
<tr>
<td>Pregnant 1st AI</td>
<td>44</td>
<td>36</td>
</tr>
</tbody>
</table>
TIME IN THE TRANSITION PEN

Nutritional management in the transition period also affects reproductive performance, not only with items such as feed bunk management to deliver the desired nutrients to cows; but also in the amount of time that a cow spends in the transition pen. A unique prospective field trial was conducted and reported by Degaris et al. (2008, 2010). Three herds (n = 993) were fed similar transition diets and dietary components. Diets were all formulated for negative DCAD with 700 g/cow/d BIO-CHLOR® (Church & Dwight Co., Inc., Princeton, NJ) and contained Rumensin® (Elanco Animal Health, Indianapolis, IN) at 1240 mg/cow/d. After following cows through confirmed pregnancies, the animals were evaluated by the number of days they were in the transition pen prepartum. Notable results shown in Figures 2, 3, and 4 include:

- Milk yield corrected for 4 % fat and 3.2 % protein was optimized when cows spent more than 21 d in the transition pen (Figure 2);
- Cows with more than 20 d in the transition pen had significantly lower calving-to-conception intervals (Figure 3);
- Risk of culling and death were significantly lower for cows spending more than 20 d in the transition pen (Figure 4).

We can certainly speculate that results may be due to increased MP intake for the transition cows in the pen for longer times or fed a negative DCAD for a longer time prepartum. However, it seems obvious from the cited study that having a distinct ration designed for transition cows for a minimum of 20 d was beneficial for reproductive performance as well as production and health.

METABOLIC DISEASES

Any and all of the metabolic diseases that can affect cows prior to an established pregnancy can logically affect reproductive processes and outcomes. Many of these metabolic diseases are closely linked to nutrition and nutritional physiology. There have been quite a few reviews on the subject of nutrition and metabolic disease even citing path analyses and odds ratios in various scenarios (Curtis and Lean, 1998; Burke et al., 2010).

![Figure 2](image-url). Four-day moving average 4.0 % fat, 3.2 % protein, corrected milk yields for cows spending various lengths of time in the transition pen (data corrected for lactation number, farm, calving order; Degaris et al., 2008).
The Dairy Cattle Reproduction Council does not support one product over another
and any mention herein is meant as an example, not an endorsement.

Figure 3. Calving to conception interval for cows spending various lengths of time in the prepartum transition pen (Degaris et al., 2010).

Figure 4. Death and culling for cows spending various lengths of time in the prepartum transition pen (Degaris et al., 2010).
Even though they might be relatively far removed from conception, it is certainly logical to associate dystocia, milk fever, retained fetal membranes, metritis, ketosis, etc., with reproductive outcomes. Two issues that we face and have to be aware of are:

- Many of these diseases have a subclinical component that we have not been able to fully evaluate regarding their effects on reproduction (or production and health);
- Many times we use blood metabolites, such as non-esterified fatty acids (NEFA) or beta-hydroxybutyrate (BHB), to assess issues related to reproduction without considering the reason for the increases or decreases in these parameters. Metabolites like NEFA have been associated with reproduction (Curtis and Lean, 1998). Certainly nutrition can directly affect these metabolites; but an intervening disease, clinical or subclinical, can also impact blood metabolites and the ideal reaction nutritionally may not be the same. For example, high blood NEFA may be associated with a reduction in reproductive performance but can be caused by feeding too much and the wrong types of fatty acids or can be due to excessive negative energy balance. The nutritional reaction to these 2 causes may be very different.

THE CASE FOR MODELING EFFORTS IN REPRODUCTION

Why Do We Need to Model?

From the discussion above, it is obvious that individual nutrients and metabolic perturbations can directly impact reproductive processes and outcomes. Many of these nutrients, blood metabolites, and diseases have individually been found to significantly affect reproduction. However, with reproductive outcome (i.e., a calf on the ground) as a multifactorial process with any of the factors being affecting by nutrition and disease, how can we more easily discover the bottlenecks on a farm to improve reproductive performance? Moreover, how can we define the most severe bottlenecks to remove those first?

First we must accept that while certain nutrients directly impact reproduction by serving a function within a reproductive process (like EFA serving as PG precursors), most of the time the way that nutrient imbalances and deficiencies impact reproduction is by setting up a cascade of events that lead to reproductive difficulties. Using the discussion on prepartum MP above as an example, it is not the lack of MP prepartum that directly impacts reproduction – rather the lack of prepartum MP likely reduces an animal’s ability to cope with various stressors, including disease and production performance that cascade to reproductive difficulties at a time that is somewhat removed from the prepartum nutritional insult. Our reactions in this case are to treat the diseases and overfeed protein in an attempt to overcome the underlying problem and these reactions can lead to additional bottlenecks related to reproduction (i.e., high BUN, reduced feed intake leading to excessive negative energy balances, elevated NEFA, etc.). Having an integrated model that is designed to look at nutrition and reproduction over a reasonable time frame will allow us to identify risk factors associated with reproductive failure and actually allow us to categorize them as to first, second, third, etc., limiting. In other words, to allow us to de-bottleneck the process. The reason we need this model is because the ability of an animal to reproduce is not only multifactorial, but also is the result of a fairly long time frame that starts at the animal’s birth (if not at conception).

Is There Enough Published Information to Put This Model Together?

At first, this modeling effort can seem like a daunting task, but there are research groups throughout the world trying to structure an overall model and others working on smaller bits and pieces of the model. There is a logical causal web of understanding the impacts of metabolism, genetics, and management on the reproductive process. Figure 5 is an overall example of this web starting with the genetic merit of the animal at the time of conception and culminating with the maintenance of a fetus. Certainly the genetic aspects of fertility will take a longer time to unravel and be modeled, but work looking at this aspect is already underway as suggested by Chagas et al. (2007). We can already superimpose the disease aspects that should be integrated upon this web of understanding as the literature has many examples of disease/metabolic path analyses that impact reproductive performance (Curtis and Lean, 1998; Curtis et al., 1985).
Mechanistic models already exist describing the relationships between nutrients, hormones, signaling factors, and metabolites with production performance. The most advanced of the models is still MOLLY, developed under the direction of the late Lee Baldwin (Baldwin et al., 1987a, b; Baldwin et al., 1994; Baldwin, 1995). Since many aspects of this model describe the signals that participate in reproductive events, the integration appears as a logical step. Not only do nutrients and their fluxes affect hormones and signaling factors related to reproduction, but the same hormones and signaling factors affect nutrient fluxes (McNamara, 2010).

Quite a bit of recent activity in the modeling effort is already published or in press (Celi et al., 2010; McNamara, 2010, 2011; McNamara et al., 2011). The efforts can certainly be moved along faster and with greater accuracy and precision as more literature is published investigating nutritional interventions and their effects on ANY reproductive endpoint or any of the intervening steps in metabolism, immunity, or diseases. These endpoints or intervening steps need not be the primary focus of the research, but we can be making these measurements at little additional cost to the research project. Publishing the results makes the information available to the modeling teams and those who research the epidemiological aspects of reproduction in Meta analyses and regression.

**CONCLUSION**

It is obvious that nutritional status affects reproductive outcomes. These effects are not only important at the time of reproductive events (cyclicity, ovulation, fertilization, etc.) but are at least as important, if not more so, as far back as calfhood. In that regard, the only way that we are going to pinpoint the nutritional bottlenecks to reproductive problems in a stepwise and logical process, without randomly trying to pinpoint these bottlenecks, is to apply modeling techniques to the process. It is not that our random processes do not work – many herds have experienced improved reproductive success with certain interventions and nutritional modifications. However, when we apply these interventions, that are known to impact reproduction, and the improvements are modest or non-existent it is not that the intervention was wrong; more likely, the intervention was not the most limiting factor in the reproductive process.

Superimposed on all of this is the phenomenon of lactational infertility (Robyn et al., 1985; McNeilly et al., 1985) which is well documented in humans.
Lactational infertility is likely an evolutionary adaptive mechanism whereby the control factors to ensure lactation are contraindicated for reproduction for a period of time postpartum. In spite of what appears to be an arduous and daunting task we should still take solace that with all of the potential issues impacting reproduction that we still have the ability to get cows pregnant, even if at a rate slower than we desire. As research progresses and our models become more integrated we should be able to identify issues at the cow and herd levels that will allow us to make large strides in improving reproductive performance.

ACKNOWLEDGEMENTS

The assistance, wisdom and data from John McNamara, Ian Lean and Jose Santos is greatly appreciated in preparing this manuscript and the slides used in the presentation.

LITERATURE CITED


