

RAPIDLY RESOLVES INFLAMMATION TO RETURN TO BALANCE



## THE CASE FOR FEEDING EPA/DHA OMEGA-3 AS AN ESSENTIAL NUTRIENT



#### What impact would extending the average lifespan of your herd(s) by one year have on farm profitability? That may seem difficult to imagine given the pressures to replace animals and bring in new genetics.

Use of sexed semen, the lure of improved genetics in a new heifer and increasing health and reproductive challenges as cows age make replacing older cows at a quick pace tempting. Next levels of profitability in dairy are dependent on improving the health span of dairy cows...helping cows to stay in herds longer because they remain healthy and highly productive.

This white paper was written because we at Virtus Nutrition believe that EPA/DHA omega-3 is an essential nutrient that has largely been overlooked in dairy, and that the evidence is clear that incorporating these fatty acids into dairy diets at a wide range of feeding rates simply makes cow health and performance better. While most of the research in dairy has been centered on reproductive and immune effects, the emerging research areas of epigenetic effects on offspring and effects of improving EPA/DHA in colostrum points to generational effects that we have yet to truly quantify.

We hope this research review gives you a look at the work that has been done already on EPA/DHA and how the research supports feeding rumen protected EPA/DHA across a wide range of feeding rates...from a basic level that supports her essential functions to higher levels for greater performance gains.



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## Contents

Research Timeline	4
Executive Summary	6
Meeting Her Essential Requirement	7
Feeding for Performance Beyond Her Requirement	16
Practical Application for Feeding EPA/DHA	18





4 EPA/DHA Research Timeline

#### PRODUCTION & REPRODUCTIVE EFFECTS

#### **Bilby, 2006**

Not only did feeding Ca salts of EPA/DHA increase milk production, but it also altered gene expression of IGF-I in the endometrium and metabolic hormones in a manner beneficial to pregnancy.

#### REQUIREMENT DEFINED

**Calder**, 2013 Established essential rate for EPA/DHA Omega-3 at 10 mg/kg.<sup>75</sup> in mammals.

#### EMBRYO EFFECTS

#### Sinedino, 2017

Feeding 10 grams of algal DHA increased total pregnancies and resulted in return to pregnancy 21 days sooner (absorbed DHA est. at ~1 g).

#### **EMBRYO EFFECTS**

#### Silvestre. 2011

EPA/DHA increases early embryo survival resulting in more pregnancies (+7 points early conception rate, reduced early aborts from 11.8% to 6.3%)

#### **REQUIREMENT DEFINED**

Staples, 2014 Established requirement for 18:2 Omega-6 at 45 mg/kg<sup>.75</sup>

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Santos, 2005

EPA/DHA embryo survival pregnancy loss from 12% to 3.2%)

EMBRYO EFFECTS

#### Moussavi, 2007

Results demonstrated that dietary supplementation with fish meal or Ca salt of EPA/DHA in early lactation significantly increased milk yield and DMI with no change in milk composition.

#### PRODUCTION EFFECTS

Greco. 2013 Linear milk increase with EPA/DHA at 2.7 lbs/7.5 g EPA/DHA up to 30 grams.

#### PRODUCTION EFFECTS

#### Calder, 2013 Typical high omega-6 diets reduce the elongation capacity of 18:3 to bioactive EPA (20:5) and DHA (22:6) due to competition for enzymes.

**REQUIREMENT** DEFINED

#### Greco, 2015

Feeding a diet with more Omega-3 and less Omega-6 attenuated the acute phase inflammatory response after intramammary LPS challenge.

IMMUNE EFFECTS

#### Oseikria, 2016

significant increase in day 7 oocyte development with just 1  $\mu$ M of DHÁ

#### EMBRYO EFFECTS

#### Ribeiro, 2016

EPA/DHA involved in elongation of conceptus, stored in high concentration in lipid droplets surrounding oocyte

#### EMBRYO EFFECTS

## **Executive Summary**

The goal of this review is to provide evidence regarding the essential requirement for EPA/DHA omega-3 in dairy diets. The research and application for essential EPA/DHA are divided into two parts: Feeding to Meet Her Essential Requirement and Feeding for Added Performance.

While the research and recommendations historically have been focused on performance level feeding rates (.1 lb to .4 lb of Strata, calcium salt of EPA/DHA), this recent review of the research shows substantial evidence for feeding EPA/DHA at basic levels (.066 lb Strata) to meet her requirement for healthy biological function, especially reproductive and immune support.

The essential absorbed rate for EPA/DHA is 10 mg/kg<sup>.75</sup> body weight (BW) (Calder, 2013). When calculating the feeding levels for essential EPA/DHA in dairy cattle, the biohydrogenation and absorption rates need to be considered. Thus, the as-fed feeding guidelines are 0.4 g EPA/DHA per 100 pounds of BW for less than 1,000-pound animals and 0.32 g EPA/DHA per 100 pounds of BW for greater than 1,000-pound animals. When feeding a Ca salt of EPA/DHA (Strata), this translates to 2.2 g per 100 pounds of BW for less.

Research has also shown added performance improvements from higher feeding rates of EPA/DHA. The increase in early lactation milk production equates to 2.7 pounds of ECM per every 7.5 grams of EPA/DHA added, up to 30 grams (Greco, 2013). Those early lactation production gains carry through the entire lactation when EPA/DHA is supplemented for the first 100 DIM (Garcia, 2015). The reproductive impacts measured at the performance levels are also substantial with increases in early conception rate and up to 50% reduction in early aborts, resulting in more retained pregnancies (Silvestre et al., 2011, Greco et al., 2013).

Fatty Acids are the last major nutrient category to truly be balanced in dairy diets. While fatty acids make up a small percentage of the ruminant diet, they are a concentrated energy source that are necessary to support the increased levels of energy corrected milk achieved in today's dairy herds. Other fatty acids, primarily Omega-6s and Omega-3s, are bioactive and support a wide range of biological functions, including immune and reproductive processes. With genetic capacity continuing to accelerate, improving the balance of fatty acids delivered to the small intestine for absorption and utilization by the cow has significant merit not only in supporting higher levels of milk production, but also improving animal welfare, reproduction, immune health, and longevity.



Necessary; extremely important

EPA/DHA serves a wide range of roles in the cow's basic biology, including supporting proper embryo development, maintenance of pregnancy and offsetting inflammatory omega-6s to resolve inflammation and protect against chronic inflammation

#### Performance

#### Accomplishing an action, task, or function

At higher feeding rates, EPA/DHA has significant impacts on improving embryo retention and pregnancy outcomes, as well as improving ECM early lactation through higher dry matter intakes and by sparing energy from the immune system (antiinflammatory effects), thus improving ECM/DMI efficiency.



## Meeting Her Essential Requirement

Fatty acids can be categorized as essential and non-essential, with essential fatty acids defined as required in the diet since the cow's body will not synthesize them on her own.

As early as 1929, linoleic acid (C18:2 Omega-6) and linolenic acid (C18:3 ALA Omega-3) were defined by Burr and Burr as essential to the diet in mammals. The saturated fatty acids, palmitic (C16:0) and stearic (C18:0) are considered non-essential, as they can be synthesized in the rumen from short chain fatty acids and elongated in the mammalian tissues. Research (Miles and Calder, 2007) defined the mono-unsaturated oleic fatty acid (C18:1) also as non-essential.



Burr and Burr, 1929 Defined 18:2 Omega-6 and 18:3 Omega-3 as essential to the diet in mammals

## Defined Requirements of Essential Fatty Acids

# The essential fatty acids are also commonly referred to as Omega fatty acids, with Linoleic (18:2) as the Omega-6 fatty acid, and ALA (18:3), EPA (20:5) and DHA (22:6) as the Omega-3 fatty acids.

Omega-6s comprise about 50% of the fatty acids in vegetable sources that are typical in dairy diets, such as corn, corn silage, cottonseed and soybean meal, and is thought to be sufficient in most dairy diets. The possible exception is during the low intake, pre-partum period leading up to parturition. The established requirement for 18:2, Omega-6 is 45 mg/kg<sup>.75</sup> BW (Staples, 2014).

On the other hand, omega-3 fatty acids are found in much lower quantities in dairy diets, with primary sources being hay, grass silages, flax seed, fish meal and Ca salts of EPA/DHA. While the very long-chain bioactive omega-3 fatty acids, EPA and DHA, are not technically considered essential, Calder (2103) argued that our typical high omega-6 diets reduce the elongation capacity of 18:3 to bioactive EPA and DHA to less than 1% (Jenkins, 2016, personal communication), thus establishing the minimum dietary requirement for EPA/DHA at 10 mg/kg<sup>.75</sup> BW.



about 50% of the fatty acids in vegetable sources that are typical in dairy diets, such as corn, corn silage, cottonseed and soybean meal, and is thought to be sufficient in most dairy diets.

## Established Requirement for Omega-6: 45 mg/kg<sup>.75</sup> BW



Established Requirement for EPA/DHA Omega-3: 10 mg/kg<sup>.75</sup> BW (Calder, 2013)

## The Research Road to Increasing Absorbed EPA/DHA

As bioactive fatty acids, EPA/DHA have roles that are beyond simply providing energy. These fatty acids are integrally involved in a wide range of biological functions, including embryo development, cell signaling, immune regulation and energy utilization. In the early 80's, Dr. Bill Thatcher from the University of Florida started down the research path that led to calcium salts of EPA/DHA being commercialized for dairy cattle feeding. He identified early embryo loss in dairy cattle as a serious problem for dairy profitability (Thatcher, 1984), with EPA/DHA as a possible remedy. There were two proposed mechanisms that became the focus of their research: 1) Insufficient interferon-tau (IFN $\tau$ ) production by the developing embryo and 2) high spikes of prostaglandin F2 $\alpha$  (PGF2 $\alpha$ ) causing the CL to regress.



Figure 1. Staples and Thatcher, 2001

EPA/DHA has a direct effect

(increases IFN $\tau$ , thus reducing

PGF2a) and improves uterine

on embryo development

environment by reduced

inflammation, also lowering

PGF2a to maintain pregnancy

PGF2a

EMBRYO

IFNτ

#### 1.EPA/DHA Link to Interferon-tau

## The first mechanism proposed was that the levels of interferon-tau (IFN $\tau$ ) production

#### by the developing embryo were insufficient. IFN $\tau$ is the primary pregnancy hormone for the dairy cow, and it is secreted from the developing embryo. In 1997, Dr. Thatcher established that levels of IFN $\tau$ produced by the developing embryo are in direct proportion to embryo's size and development, and that IFN $\tau$ lowers mRNA synthase

for PGF2 $\alpha$  (Thatcher et al., 1997).

## 2.High Levels of PGF2a Regressing CL

The second mechanism considered to be affecting embryo losses was high levels of PGF2 $\alpha$  in the endometrium tissue causing the established CL to be regressed. Mattos et al., 2003 reported a linear decline in PGF2 $\alpha$ secretion as IFN $\tau$  is increased, connecting the dots between embryo growth and maintenance of pregnancy. He also reported a significant reduction in PGF2 $\alpha$  when 3 $\mu$ M EPA/DHA was added (Figure 2). This research helped establish that IFN $\tau$  and EPA/DHA both act to reduce PGF2 $\alpha$  through independent and separate pathways.

Once Dr. Thatcher and team established these mechanisms that could be affecting pregnancy loss, they moved on to doing various dose studies to better understand what levels of EPA/DHA might be needed to have a substantial effect on pregnancy maintenance. In 2002, Mattos et al. conducted an experiment feeding fish meal to supply varying gram levels (12.8g, 24.1g, or 54g) of EPA/DHA to measure ovarian effects. All feeding levels reported significant reduction in PGF2 $\alpha$  when cows were challenged with oxytocin compared to the control diet with no added EPA/DHA (Figure 3). Figure 2. Mattos et al., 2003

EPA/DHA Omega-3

## $\uparrow$ IFN- $\tau$ = Linear CL Retention Across Range of EPA Omega-3



Larger embryos release greater quantities of IFN $\tau$ , thus having greater impact on reducing PGF2 $\alpha$  & corpus luteum retention. Figure 3. Mattos et al., 2002



Cows fed a less inflammatory diet with EPA/DHA had a lower PGF2 $\alpha$  spike vs. cows on a traditional control diet.

As a follow-up study in 2003, Mattos et al. performed a dose titration study where 18 grams of EPA/DHA (40  $\mu$ M) were determined to have the maximum effect on reducing PGF2a to support pregnancy maintenance. Thus, the original feeding recommendation of 18 g (1/4 lb. of Strata) was decided and became the primary feeding rate for future reproductive studies and on-farm implementation.

## Embryo Effects at Low Levels

#### While the work done at the University of Florida laid a great foundation for understanding the role that EPA/DHA plays in pregnancy maintenance, more recent work tested the impact of these essential nutrients on embryo development at very low levels.

Research from Oseikria et al. (2016) reported that just 1 micromolar ( $\mu$ M) of DHA significantly increased day 7 conceptus development and cell division in an IVF model (Figures 4 & 5). To put things in perspective, the research from Oseikria was testing levels of DHA that were just 1/40th of the established feeding recommendations by University of Florida. This research helps define just how essential EPA/DHA is in improving early embryo growth which leads to improved pregnancy retention (greater embryo growth means increased IFN $\tau$  production that reduces PGF2 $\alpha$ , thus improving pregnancy recognition and maintenance of the corpus luteum).

Figures 4 & 5. Oseikria et al., 2016





Even at very low levels of DHA (1  $\mu$ M or .45 g), significant improvements in embryo development were seen at the day 7 blastocyst stage, as well as a positive trend in # of cells per blastocyst in vitro.

Meanwhile, at the University of Guelph, Dr. Eduardo Ribeiro continued the work he started when he was at Dr. Jose Santos' lab at UFL as a grad student. Ribeiro (2016) helped define the essential role that EPA/DHA plays in elongation of the early conceptus, showing how EPA/DHA are stored in high concentrations in lipid droplets surrounding the oocyte to be drawn upon in early phases of development. Ribeiro's work adds to the importance of EPA/DHA in establishment and maintenance of early pregnancy and provides important information on the levels that are required to support the positive effects when absorbed in the endometrium.

Impact on pregnancy outcomes at lower levels of DHA were explored by Sinedino et al., 2017 (Figure 6). His research showed that feeding 10 grams of algal DHA increased total pregnancies and resulted in a return to pregnancy 21 days sooner. In addition, there was greater gene expression (RTP4) for IFN $\tau$  in DHA-fed pregnant cows (Figure 7). Algal DHA has been shown to have a high biohydrogenation rate at 90% (Vlcek et al., 2017) based on incorporation into milkfat. Thus, the absorbed DHA in this study was estimated to be at ~1 gram. This research helps reinforce the positive biological functions of EPA/DHA at feeding rates closer to what was reported by Calder (2013) at 10 mg/kg<sup>.75</sup> BW per day to meet the essential need in mammals.

**Mattos**, 2002 Showed reductions in PGF2α from fish meal across range of EPA/DHA supplementation levels (12.8 to 54 g).

**Mattos**, 2003 Showed linear decline in PGF2a from IFN $\tau$ increase (greater embryo size). Also saw decline in PGF2α when 3 μM EPA/DHA added.



Figure 6. Sinedino et al., 2017

Significant improvements in rate of pregnancy seen when DHA omega-3s fed vs. cows with no supplemental omega-3s.

Day Postpartum

#### Figure 7. Sinedino et al., 2017 nterferon-τ Receptor Protein (RTP4)



Gene expression of Interferon- $\tau$  receptor protein increased with low levels of DHA, reinforcing the positive effects of EPA/DHA at essential levels.



The recent work from Oseikria (2016), Ribeiro (2016), and Sinedino (2017) prompted a retrospective look at the EPA/DHA dose titration study initially performed at the University of Florida. In hindsight, this foundational EPA/DHA study by Mattos et al. (2002) showed a significant reduction in PGFM at the lowest rate of 12.8g (2.6% fish meal) EPA/DHA, indicating high bioactivity at a lower dose. In addition, the EPA/DHA dose response curve (Mattos, 2003) which was used to establish the 18-gram feeding rate recommendation showed significant effects all along the dose curve, not just at the point on the curve where the 'maximum effect' was established (Figure 8).

In retrospect, what was not considered when establishing the initial feeding recommendations was two-fold: 1) the biological significance that IFN $\tau$  plays in pregnancy as the primary pregnancy signaling by the embryo and 2) the role EPA/DHA plays in early embryo growth, thus increased IFN $\tau$ production in the early days after fertilization. Adding EPA/DHA at the essential level of 10 mg/kg<sup>.75</sup> BW (Calder, 2013) helps meet this basic biological need to support improved pregnancy outcomes.

#### Figure 8. Mattos et al., 2003

EPA/DHA Reduces PGF2α (to Improve Pregnancy Retention)



While the original Ca salt with EPA/DHA (Strata) was formulated at the maximum impact on reducing PGF2a (18 grams/40  $\mu$ M), the data shows significant results at much lower levels of EPA/DHA. (4.5 g = .06 lb Strata, 9 g = .12 lb Strata, 18 g = .25 lb Strata)



## EPA/DHA Role in Immune Regulation

The biology of EPA/DHA Omega-3 needs to be explained in the context of Omega-6s, since these fatty acids counterbalance each other from an immune perspective. Omega-3s are anti-inflammatory, while Omega-6s have pro-inflammatory effects on immune function. Once absorbed at the small intestine, omega fatty acids are stored in the phospholipid layer in every cell in the cow's body, with the exception being red blood cells. From there, they are drawn upon by the cow for many essential biological functions, including synthesis of eicosanoids, cell signaling, improving membrane fluidity, strengthening tight junctions in intestinal lining, and embryo development to name a few.

It is very common to have an imbalance of omega-6 and omega-3 at the tissue level in dairy cattle. This imbalance occurs in part due to the competition for key enzymes that are utilized in the elongation and desaturation of both the Omega-6 and Omega-3 pathway (Calder and Miles, 1998). Typical dairy diets are high in omega-6s (from corn, corn silage, distillers, cottonseed) and low in omega-3s (dry hay, grass silage, flax), creating competition for these shared enzymes that limit conversion of ALA omega-3 to the bioactive forms of omega-3: EPA and DHA. This imbalance increases the support of inflammatory pathways and lessens the cow's ability to resolve inflammation quickly (Figure 9).



14 Meeting Her Essential Requirement

## EPA/DHA Derived Resolvins & Protectins

While inflammation is a necessary and natural response to an insult, excess inflammation is a problem. When inflammation is slowly resolved, it leads to chronic inflammation, declined health, lower productivity and reproductive outcomes.

Discovered by Dr. Charles Serhan (Harvard, 1994), resolvins and protectins are specialized immune molecules (derived from EPA/DHA) that drive the resolution of inflammation and prevent the immune system from over-responding. When a cow experiences an insult, whether it be from stress, infection, injury or diet-induced, neutrophils rush in as the first responders. Resolvins and protectins act as the 'brakes' that prevent too many neutrophils from responding (anti-inflammatory). They also actively resolve inflammation by signaling macrophages to respond, cleaning up the damaged tissue and restoring balance (pro-resolving) and return to homeostasis. Serhan's discovery of resolvins and protectins led to a better understanding that the resolution of inflammation is an active process that needs to be biologically supported, as it does not simply happen by default (Figure 10). These EPA/DHA derived immune molecules are essential to supporting balanced immune function and creating a healthy uterine environment for the early developing embryo.

#### Figure 10. Adapted from Serhan, 2014

(C18:2 Omega-6)

## Role of Lipid Mediators in Inflammation and Resolution

Specialized pro-resolving lipid mediators derived from

EPA/DHA are generated during the resolution phase and control early events in acute inflammation by

protecting against excess inflammation.



Lipid Mediator Class Switching Acute Inflammation Resolution Homeostasis Leukotrienes Maresins Lipoxins **Resolvins & Protectins Prostaglandins** Specialized Pro-Resolving Mediators (SPMs) Arachidonic Acid (C20:4) Eicosapentaenoic Acid - EPA Docosahexaenoic Acid - DHA (C20:5 Omega-3) (C22:6 Omega-3) Linoleic Acid

## Feeding EPA/DHA for Performance

While the evidence is strong for supplementing EPA/DHA at a base level to meet her essential requirement, the initial research that created a foundation for use on dairy farms was at a higher feeding rate, resulting in performance gains in ECM, production efficiency and reproductive outcomes.

With 10-plus years of on-farm results, the benefits are compelling in dairy herds where grouping and logistics allow them to target feed EPA/DHA at performance level feeding rates in early lactation.

## Improved Reproductive Outcomes

As mentioned earlier, the base reproductive research done on calcium salts of EPA/DHA (Strata) was performed at the <sup>1</sup>/<sub>4</sub> pound feeding rate. Dr. Jose Santos (2005) conducted research while at UC Davis showing greater than 50% reduction in early embryo loss, and those results were repeated by Silvestre at the University of Florida (2008) (Figure 11). Conception rates at 28 days post-insemination were also improved compared to a Ca salt of palm oil (EnerGII) (Figure 12).

#### Figure 11. Silvestre et al., 2008; Santos et al., 2005 Reduced Pregnancy Loss from Day 32 to 60



Pregnancy losses between 1st vet check at 32 days and the reconfirm check at 60 days was significantly reduced with supplemental EPA/DHA during fresh and breeding period.

#### Figure 12. Silvestre et al., 2008 Higher 1<sup>st</sup> & 2<sup>nd</sup> Service Conception Rate



Higher conception rates from increased fertility and improved early embryo development with EPA/DHA

## Linear Milk Response to EPA/DHA

# While improving reproduction has been a primary driver of the research on EPA/DHA, the positive effects on milk production became a valuable outcome. When reproductive research was performed, milk outcomes were often recorded as well.

After several studies with similar responses in ECM (Bilby, 2006; Moussavi, 2007; Silvestre 2011), milk responses to EPA/DHA became a specific area of research interest. Greco et al. (2013) performed research that altered the ratio of omega-6 to omega-3 by feeding varying levels of calcium salts of EPA/DHA (Strata). What resulted was a linear increase in ECM in early lactation at 2.7 pounds of milk per 1/10 pound of Strata fed. This research was followed up by Garcia et al. (2014) showing a high degree of carryover ECM over the rest of lactation when EPA/DHA is fed out to 105 days in milk.

When the data was normalized (Bilby, 2006; Moussavi, 2007; and Greco, 2015), the summary reinforces the linear milk production response when EPA/DHA is added to early lactation diets starting just after parturition (Figure 13).

#### Figure 13. Adapted from Bilby, 2006; Moussavi, 2007; Greco, 2015

#### Ca salt of EPA/DHA Omega-3 Effects on Milk Production



Strong linear milk response has been shown across multiple studies from supplementing EPA/DHA early lactation.

#### Milk Response 2.7 lb ECM/7.3g EPA/DHA fed (.1 lb Strata / .5 lb EnerG-3)

## Practical Application of EPA/DHA in Dairy Diets

# While the research clearly underscores the essential role EPA/DHA plays in dairy cow reproductive and immune biology, it is often logistics and economics that determine the best way to apply the science.

In the case of EPA/DHA, the research spans a wide range of feeding rates, showing value across that spectrum from very low levels of EPA/DHA to the higher performance feeding rates that have been commonly fed in early lactation.

Virtus Nutrition has two products in their line-up of calcium salts of EPA/DHA: Strata is a concentrated source with 16% EPA/DHA, and EnerG-3 is designed to deliver all three key fatty acids in one product, with Palmitic (59%), Oleic (25%) and a lower concentration of EPA/DHA (3.2%). While both products can be fed to meet the cow's essential requirement, Strata's concentration of EPA/DHA allows for a low inclusion rate that can be fed alongside a herd's primary fat supplement and/or added to a mineral pack, whereas EnerG-3 is generally fed as the main fat supplement that covers the bases for both EPA/DHA plus energy from the other fatty acids. Strata is also generally fed at higher feeding rates after calving to increase early milk, resulting from greater anti-inflammatory support post-calving.

Feeding to Meet Her Essential Requirement	Feeding EPA/DHA for Performance
<b>Lactating Dairy Cows</b> .066 lb/day of <b>Strata</b> or .33 lb <b>EnerG-3</b>	Lactating Dairy Cows .2–.4 lb/day Strata early lactation
<b>Pre-Fresh Dry Cows</b> .066 lb/day of <b>Strata</b> Plus .22 lb <b>Prequel</b> with Omega-6s Or .2 lb <b>EnerG-3</b>	.1–.15 lb <b>Strata</b> for one group TMR or longer DIM pens
<b>One Group Dry Cow</b> .066 lb/day <b>Strata</b> plus .11 lb <b>Prequel</b>	.5–1.25 lb <b>EnerG-3</b> can be fed across all stages of lactation
Breeding Heifers .044 lb/day of Strata	
Rule of Thumb for Essential Rate Calculations 2.2 g Strata/100 lb BW for Lactating and Dry Cows 2.6 g Strata/100 lb BW for Heifers	

18 Practical Application of EPA/DHA in Dairy Diets

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