ABSTRACT

SMALL, DUSTI JEANNE VANDERWENDE. Effect of Feeding Supplemental Rumen-Protected Niacin (Niashure™) on Milk Yield and Milk Composition in Early Lactation Holstein Cows. (Under the direction of Dr. B. A. Hopkins and Dr. L. W. Whitlow.)

Eighty-six multiparous Holstein cows were assigned to one of three treatments to investigate the effects of 0, 6, and 12 g/d supplemental rumen-protected niacin (Niashure™) from 35 to 142 DIM. Cows received a corn silage based total mixed ration fed *ad libitum* twice daily through individual Calan® feeding stations. At the morning feeding all cows received 21 g/d of a topdress composed of a bentonite carrier blended with treatments of 0, 6, or 12 g/d Niashure™. Feed intakes were recorded daily. Analysis of weekly feed samples composited monthly indicated that diet dry matter (DM) contained 18.3% crude protein (CP) and 21.5% acid detergent fiber (ADF). Milk yields were recorded at each milking (2x/d). Weekly milk samples composited from consecutive morning and afternoon milkings were analyzed for fat, protein, urea nitrogen, and lactose. High and low ambient temperatures and humidity levels in the free-stall barn were recorded daily. Skin temperatures were measured at the rear udder attachment five days per week at the p.m. milking using an infrared thermometer. Body weights and body condition scores were measured weekly. Data were analyzed using the mixed procedure of SAS® and significance was declared at $P < 0.05$. There were no significant treatment effects on skin temperatures, body weights, or body condition scores. Milk yield (kg/d), fat (%), protein (%), lactose (%), MUN (mg/dL), and DMI (kg/d) were (38.4, 4.0, 2.9, 4.8, 21.1, 24.9), (40.2, 3.9, 2.8, 4.7,

21.3, 24.9), and (40.0, 4.0, 2.9, 4.8, 21.3, 24.5), for treatments of 0, 6, and 12 g/d Niashure™, respectively, with no significant treatment effects. In comparison to control, Niashure™ supplementation of 12 g/d tended to improve feed efficiency (kg milk / kg DMI) from 1.58 to 1.65 ($P < 0.08$). In comparison to control, supplementation of 6 g/d Niashure™ significantly improved feed efficiency from 1.58 to 1.69 ($P < 0.02$).

Effect of Feeding Supplemental Rumen-Protected Niacin (Niashure™) on Milk Yield and Milk Composition in Early Lactation Holstein Cows

by
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A thesis submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the Degree of
Master of Science

Animal Science

Nutrition

Raleigh, North Carolina

2010

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BIOGRAPHY

Dusti Jeanne Vanderwende was born in Greenwood, Delaware, on January 5, 1985, to Mr. and Mrs. Douglas Edward Vanderwende. She grew up in the midst of a large family farming operation that included commercial grains, broiler chickens, vegetables, hay, and Holstein dairy cows. She was active in school, sports, church, and community service organizations, particularly her local 4-H club. She began breeding registered Suffolk and Lincoln Longwool sheep at age six as a 4-H project, and continued to be very active in the Lincoln show circuit until 2008.

Dusti graduated from Woodbridge High School in 2003. Her interest in the inherited traits of her sheep led her to the University of Delaware where she became enrolled in the Science & Engineering Scholars program. She worked in an animal science immunology lab under Dr. Robert Dyer where she completed research and an undergraduate thesis entitled, “Toll-Like Receptor Expression and Stimulation in the Sensitive Lamina of the Bovine Hoof.” She graduated from the University of Delaware in 2007, having earned a Bachelor’s Degree of Distinction in Animal Science with a concentration in Biotechnology, and minors in Biology and French. Later that year, Dusti enrolled in the Master of Animal Science and Nutrition program at North Carolina State University under the direction of Dr. Brinton A. Hopkins and Dr. Lon W. Whitlow.

Dusti is currently a member of the Class of 2013 at the College of Veterinary Medicine at North Carolina State University, and is looking forward to becoming a food animal practitioner. She was married to John H. Small, Jr., of Greensboro, North Carolina, on May 30, 2009.

ACKNOWLEDGEMENTS

I first must offer my deepest gratitude to Dr. Hopkins for introducing me to the wonderful state of North Carolina, and for his leadership, patience, and attention to detail throughout my research and writing of my thesis. He was also a strong voice in my decision to apply to and accept my invitation to attend vet school at North Carolina State University, and for that I am grateful. I also thank Dr. Whitlow for his advice throughout our trial and encouragement to always dig a little deeper into the research, while keeping sight of the big picture. I hold you both in the highest regard, and I especially admire you for your contributions to improving dairy farms in North Carolina and working with youth across the state to cultivate understanding and respect for agriculture.

My research would not be complete today without assistance for Shannon Davidson. From showing me how to use a Wiley Mill®, to becoming my personal SAS® consultant, she was there every step of the way to provide insight and direction. I cannot imagine what I would have done without her and wish her the best of luck in her new career at North Carolina State University.

I also extend appreciation to Correll Hall at the Piedmont Research Station for his daily management and oversight of data collection during our trial, as well as his friendship. Correll along with Andy, John, Jason, and other staff members always seemed eager to help me bleed cows and review data. Franco also provided immeasurable assistance analyzing feed samples, keeping track of blood

samples, and passing the time inside the lab with intriguing conversation. For 15 months, I looked forward to every Tuesday and my trip to Salisbury to visit my friends and sometimes even eat lunch—it was truly a pleasure working with everyone.

I also thank Marian for all her help throughout my program, especially for keeping me in line, keeping my documentation straight, her stories of Delaware, and her endless advice. It was always fun to run into her in the hall.

Last, but not least, I thank my entire family for their support through this project, for understanding when I left Christmas season festivities a few days early so that I would not miss a Tuesday at the Station, and for instilling in me a strong work ethic and desire to dedicate my life to agriculture.

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ABBREVIATIONS

The following abbreviations may be included in the text without explanation.

ADF	acid detergent fiber
BCS	body condition score
BHBA	beta hydroxybutyrate
BW	body weight
C16	palmitate (fatty acid)
C18	oleic (fatty acid)
CP	crude protein
DIM	days in milk
DM	dry matter
DMI	dry matter intake
ECM	energy-corrected milk
FCM	fat-corrected milk
NA	nicotinic acid
NAD	nicotinamide adenine diphosphate
NAM	nicotinamide
NDF	neutral detergent fiber
NEFA	non-esterified fatty acids
NPN	non-protein nitrogen
OM	organic matter
RNA	ribonucleic acid
SBM	soybean meal
THI	temperature-humidity index
VFA	volatile fatty acid

LITERATURE REVIEW

Introduction

In 1978, the National Research Council (1978) stated that niacin synthesized naturally by microbes inside the rumen was adequate for optimal dairy performance. In the 30 years since then the annual average milk yield in the United States (kg/cow/yr) has increased 60%, from approximately 5,100 to 8,599 in 2004 in the United States (USDA, 2005). It is widely speculated that the niacin requirement to support this enhanced level of production in dairy cows today is above that which can be produced naturally by the dairy cow, and that supplementation of niacin may increase her production potential. Nevertheless, efforts to supplement niacin have shown divergent results in terms of improved milk yield, milk components, blood non-esterified fatty acids (NEFA), blood beta-hydroxybutyrate (BHBA), and heat stress tolerance. This inconsistency in results may be due to the degradation of niacin by rumen microbes following ingestion (Santchi et al., 2004; Schwab et al., 2006). Recently, technology has become available that enables supplemental niacin to resist ruminal degradation and be absorbed in a biologically active form in the intestine. One rumen-protected niacin product available in the marketplace is Niashure™ (Balchem Corporation, New Hampton, NY), which is protected by a patented lipid capsule. Note that there are no studies supplementing ruminally resistant niacin until 2007, and the niacin

products used in these studies are designated “rumen-protected” to distinguish them from traditional unprotected niacin forms.

Niacin Structure and Synthesis

Niacin is a water-soluble B-vitamin that consists of a pyrimidine ring with either an amide or carboxylic acid side group attached to position 5. Side groups distinguish the two biological forms of niacin: nicotinamide (NAM) and nicotinic acid (NA), respectively. Both NA and NAM can be incorporated into nicotinamide adenine dinucleotide (NAD), which is an essential coenzyme for many oxidation reactions in energy metabolism, but NAM is the sole reactive niacin form. While NA and NAM are synthesized naturally by bacteria and plants through condensation of 3- and 4- carbon units (Brown and Reynolds, 1963), NAM can also be formed during hydrolysis of NA under acidic conditions (Campbell et al., 1994). In dairy cows, niacin is synthesized naturally in the rumen and is also consumed in grains and B-vitamin supplements.

Niacin Synthesis, Degradation, and Absorption in Ruminants

Researchers know little about the metabolic fate of dietary niacin in the rumen, but most recognize that very little is absorbed across the digestive tract.

Researchers do not clearly understand the interaction between NA, NAM, and rumen microbes. A limited quantity of ingested NAM may be absorbed by the rumen wall before the remainder is rapidly hydrolyzed to NA and ammonia in the acidic environment of the rumen and abomasum. Nicotinic acid is utilized or degraded in the rumen by bacteria and protozoa, helping to enhance microbial protein synthesis directly (Riddell et al., 1980; Riddell et al., 1981), or enhance protozoal populations that maintain rumen environments favorable for bacteria (Horner et al., 1988; Doreau and Ottou, 1996). Almost no exogenous niacin reaches the duodenum where it is expected to be absorbed, as it was shown to have a ruminal disappearance rate of 98.5% (Santchi et al., 2004). However, if exogenous niacin increases microbial growth, especially in bacterial species that synthesize niacin, then additional niacin may be available for absorption later in the digestive tract because it is associated with bacterial cells where it is protected from the harsh conditions of the rumen.

To begin elucidating the process of niacin absorption, Erickson et al. (1991) examined whether the form of niacin, NA or NAM, affected absorption from the rumens of 3 mid-lactation Holstein cows. Rumens were manually emptied, washed twice with tap water, and filled with a neutral control buffer. Treatment cows received NA or NAM in the ventral and caudoventral blind sacs of the rumen and were fasted while samples were collected every 20 minutes during the one-hour collection period. Nicotinamide was absorbed at a rate of 0.98 g/h, yet NA did not

appear to be absorbed from the rumen during a 1-hour period. It was proposed that NA was not absorbed because it was ionized in the rumen buffer at pH 7. Under natural rumen conditions with rapid fermentation and the presence of volatile fatty acids (VFAs), the lower pH would have prevented ionization and increased absorption rate of NA. In contrast, NAM is only weakly acidic so it remained stable in the buffer solution and was more readily absorbed from the rumen (Erickson et al., 1991). In 1980, Riddell et al. (1980) found that niacin itself significantly lowered pH at supplemental levels greater than 1000 ppm. While Erickson et al. (1991) indicated that NAM is more readily absorbed from the rumen than NA, the study is not conclusive because conditions inside the rumen were not natural.

To further characterize niacin absorption *in vivo*, Campbell et al. (1994) supplemented 12 g/cow/d NA, 12 g/cow/d NAM, and a combination of 6 g/cow/d each of NA and NAM to 4 multiparous Holstein cows with ruminal and duodenal cannulae beginning at 200 ± 25 days in milk (DIM). At regular intervals during each 14-day period, blood samples, urine samples, ruminal fluid, duodenal digesta, and bacterial samples were collected. Regardless of the form of niacin supplemented, only free NA was detected in the duodenal fluid and it only accounted for an estimated 17% of the niacin supplemented. Supplementation of NAM alone resulted in the greatest increase in NA concentration from control, but there was no increase in NAM concentration. Nicotinamide was not detected in

ruminal or duodenal fluids after niacin supplementation. Ruminal and duodenal NA concentrations were both significantly affected by time and treatment by time, but the concentration of NA in ruminal fluid increased and declined more rapidly than in duodenal fluid, returning to normal just 1 hour after feeding. It was also found that only NA was present in the blood plasma of the cows, and plasma concentrations were significantly higher when niacin was supplemented as NA rather than NAM. Campbell et al. (1994) suggested that since NAM was not present in ruminal fluid or blood plasma, it must be hydrolyzed to NA and ammonia very quickly after entering the rumen. If NA was the only niacin form present in ruminal fluid, duodenal fluid, and blood plasma (Campbell et al., 1994), while NAM was the only niacin form absorbed through the rumen wall (Erickson et al., 1991), then it is possible that a limited amount of NAM is absorbed before it is converted to NA in the rumen, yet even the absorbed NAM is immediately converted to NA in blood. Treatment did not significantly affect bacteria or protozoa numbers in the rumen (Campbell et al., 1994).

Santschi et al. (2004) conducted two experiments to estimate the B-vitamin synthesis in the rumen, and disappearance prior to and from within the small intestines of 4 ruminally, duodenally, and ileally cannulated, lactating dairy cows. In the first experiment, niacin (20 g/cow/d NAM) was supplemented 5 days prior to and 4 days during a collection period. It was determined that 98.5% of niacin disappeared before the duodenal cannula. Previously, a similar niacin

disappearance rate in steer calves was estimated at 93.5% (Zinn et al., 1987), but Santschi et al. (2004) noted that Zinn et al. (1987) fed the calves a concentrate-rich diet that would have affected rumen pH associated with bacterial populations and passage rate, such that the determined rate of niacin disappearance may not be representative of that in dairy cattle. Santschi et al. (2004) could not completely exclude some minimal niacin absorption through the rumen wall in their experiment, and suggested the beneficial effect of niacin supplementation in many studies was a result of its impact on rumen microflora or possibly even diffusion through the gastrointestinal wall proximal to the duodenal cannula, where measurements were made.

In the second experiment, Santschi et al. (2004) infused niacin (6 g/cow/d NAM) abomasally 1 day prior to and each day during a 4-day collection period. Duodenal flows of NAM did not increase with treatment, but NA increased by an average of 481 ± 100 mg/d. These findings agree with preceding research that indicated NAM was hydrolyzed to NA, but Santschi et al. (2004) found that hydrolysis occurred in the abomasums. Campbell et al. (1994) found that hydrolysis occurred in the rumen. Santschi et al. (2004) reported apparent intestinal absorption of NA and NAM are both highly absorbed once they reach the intestine, at 73% and 94%, respectively. They noted 7% greater NA absorption when NAM was supplemented than when there was no vitamin supplementation, and they attributed this effect to the conversion of NAM to NA after post-ruminal

infusion. Again, absorption rates coincided with preceding research in beef cattle (Zinn et al., 1987). Santschi et al. (2004) estimated ruminal synthesis of niacin to be 2.2 g/cow/d, not accounting for absorption, destruction, or use before the duodenal cannula. In contrast to Campbell et al. (1994) who suggested only NA was synthesized in the rumen, Santschi et al. (2004) found that substantial amounts of both NA and NAM were synthesized. However, Campbell et al. (1994) only examined ruminal and duodenal fluids, whereas Santschi et al. (2004) measured total ruminal and duodenal content to account for bacteria-associated niacin (Santschi et al., 2004).

Schwab et al. (2006) demonstrated that even without supplementation, duodenal flows of B-vitamins, including niacin, were higher than intake in 4 multiparous and 4 primiparous, ruminally and duodenally cannulated, Holstein cows on diets varying in forage and non-fiber carbohydrate over 21 days during mid-lactation. For diets consisting of forage to non-fiber carbohydrate (NFC) ratios of 35:30, 35:40, 60:30, and 60:40, duodenal flows of niacin were 24.6, 28.7, 19.1, and 19.7 g/d, while apparent syntheses were 4.5, 15.5, 7.2, and 13.9 g/d respectively. Authors proposed that the observed variation in synthesis of B-vitamins was affected by changes in diet composition, digesta passage rate, and populations of ruminal microbial species that may have been cross-feeding off each other. Ruminal niacin synthesis was 4- to 62-fold greater than the other B-vitamins and the vast majority of niacin reaching the duodenum was in the NA

form, yet synthesis values from cows on all diets were still lower than the 2.2 g/d estimated by Santschi et al. (2005). Schwab et al. (2006) attributed the high level of NA reaching the duodenum to the hydrolysis of NAM to NA in the acidic environment of the rumen. Authors recognized that the equation they used to calculate niacin synthesis did not account for microbial destruction, ruminal absorption, or bioavailability of feed derived B-vitamins, but no data quantifying ruminal destruction or bioavailability of feed-derived B-vitamins was available.

Interactions Between Niacin and Rumen Microbes

While some researchers focused on elucidating niacin absorption, others began exploring how niacin affects microbial populations in the rumen. *In vitro* studies conducted by Riddell et al. (1980) demonstrated that supplemental niacin (NA) significantly increased microbial protein synthesis in rumen fluids of cows consuming diets consisting of corn, corn and brome grass, or brome grass only, but not at equal rates according to diet or time. With the corn diet, maximum protein synthesis was observed at 50 ppm niacin and there was a decline in effectiveness of niacin to promote protein synthesis when it was supplemented at levels greater than 100 ppm. At all sampling times (0, 3, 6, and 12 h), niacin treatment numerically increased microbial protein levels in the rumen fluid, but the increase was only significant at 12 hours.

To follow up on prior studies, Riddell et al. (1981) conducted a series of *in vitro* and *in vivo* experiments to demonstrate the effect of niacin on rumen microbial synthesis when paired with either urea or soybean meal (SBM) as nitrogen source. *In vitro*, niacin (100 ppm) added to rumen fluid derived from a cannulated donor increased microbial protein synthesis when the donor was fed with SBM, but not with urea. *In vivo*, it was demonstrated that supplemental niacin (6 g/cow/d) increased protein percentage in the milk when fed to 24 lactating dairy cows past peak lactation, possibly as an indirect effect of increased microbial protein yield.

The only published study that defined the affinity of rumen microbes for niacin was conducted by Abdouli and Schaefer (1985). They described *Lactobacillus plantarum* and *Treponema bryantii* as two of the only bacterial species in the rumen known to require niacin. Even though niacin is essential to *L. plantarum* and *T. bryantii*, the authors found that these species were characterized by such low saturation constants (0.00066 to 0.0012 µg/ml, respectively) that they only required 0.012 to 0.022 µg/mL niacin to achieve 95% of their maximum growth rate. Other studies indicated that endogenous niacin concentrations in the rumens of cattle exceeded 0.08 µg/mL (Buziassy and Tribe, 1960), suggesting that supplemental niacin did not affect bacterial growth rate. These findings are in contrast to those of Riddell et al. (1980) and Riddell et al. (1981) that suggested niacin increased microbial protein synthesis in the rumen. However, Abdouli and

Schaefer (1985) do not account for the effect of diet on the ability of niacin to impact microbial protein growth or for utilization of niacin by protozoa, which could in turn, promote the growth of bacterial populations by removing starch in the rumen environment.

Horner et al. (1988) studied the impact of niacin supplementation on feed intake, ruminal fermentation, and nutrient digestibility in 4 mature, non-lactating, Holstein heifers during two 14-day periods. Digesta samples were collected from ruminal and duodenal cannulae during the last 3 days of each 14-day period. Niacin supplementation (6 g/cow/d) increased ruminal protozoa and ribonucleic acid (RNA) concentrations in digesta. Authors also observed significantly increased digestibility of neutral detergent fiber (NDF), but not acid detergent fiber (ADF), and attributed this shift to a change in microbial population that resulted in greater hemicellulose digestion. Additionally, niacin increased daily production of nitrogen by rumen microbes, while significantly reducing non-ammonia nitrogen from feed reaching the duodenum, suggesting that niacin caused rumen microbes to incorporate more feed nitrogen into the microbial fraction. Authors expressed concern for high producing cows that require greater amounts of rumen undegradable protein (Horner et al., 1988). Findings from Horner et al. (1988) validate increased microbial protein synthesis as a result of niacin supplementation (Riddell et al., 1980; Riddell et al., 1981) without contradicting

studies that suggest niacin supplementation does not enhance bacterial growth (Abdouli and Schaefer, 1985).

Doureau and Ottou (1996) examined the effect of supplementary niacin (6 g/cow/d NA) on digestion in 4 multiparous dairy cows fitted with ruminal and proximal duodenal cannulae, observed from 118 to 130 DIM. Although rumen bacterial populations were unchanged by niacin, there was a slight increase in purine and pyrimidic bases in the fluid-associated bacterial fraction that authors suggested indicated a higher rate of protein synthesis and growth. Niacin supplementation significantly increased protozoal numbers of *Ophryoscolecidae* between 0900 and 1400h, suggesting that exogenous niacin was inadequate to support maximal growth of protozoa under normal feed conditions since they cannot synthesize their own niacin. Microbial and non-microbial nitrogen flows were unaffected by treatment (Doureau and Ottou, 1996).

Christensen et al. (1998) found that niacin (12 g/cow/d NA) did not affect microbial protein synthesis. Researchers measured treatment effects of niacin on apparent digestibility of nitrogen in the total tract, finding that ammonia nitrogen was decreased by niacin with a low fat diet and increased by niacin with a high fat diet. Nevertheless, the lowest nitrogen level observed was sufficient for maximal microbial protein synthesis.

It seems that if niacin supplementation affected microbial populations in the rumen such that protein synthesis was affected, digestibility of carbohydrates and

fiber would be altered as well. Campbell et al. (1994) found that total apparent digestibilities of dry matter (DM), organic matter (OM), crude protein (CP), and NDF were greater for cows supplemented with NA plus NAM than cows supplemented with only NA or NAM, and Doureau and Ottou (1996) revealed that niacin supplementation increased the theoretical degradability of DM. However, in most studies niacin did not affect digestibility. In general, there were no treatment effects on rumen DM (Riddell et al., 1980; Campbell et al., 1994), OM (Doureau and Ottou, 1996; Schwab et al., 2006; Campbell et al., 1994), NDF (Schwab et al. 2006; Campbell et al., 1994) or ADF (Schwab et al., 2006; Campbell et al., 1994). This anomaly can be justified by the implication that niacin improves feed efficiency, and therefore may increase protein synthesis without altering carbohydrate or fiber digestibility.

Effect of Niacin on Volatile Fatty Acids

The balance of VFAs in a cow's rumen is important because it reflects her diet and it affects the internal balance of rumen microflora. Most studies examining the impact of niacin supplementation on VFA concentrations in the rumen found that it increased propionate relative to acetate (Riddell et al., 1980; Schwab et al., 2006). However, Christenson et al. (1998) reported that acetate increased relative to propionate while total ruminal VFA in the ruminal fluid remained constant, and

Zimmerman et al. (1992) reported no change in the ruminal acetate:propionate ratio. Schwab et al. (2006) also found that biosynthesis of niacin is negatively correlated with molar acetate percentage which could account for the increased propionate:acetate ratio reported in some studies. Doureau and Ottou (1996) found that butyrate was significantly increased with supplementation of niacin.

Supplemental Niacin and Protein

Some digestion studies suggest that niacin increased microbial protein synthesis and possibly protein available for incorporation into milk. Higher protein yields in milk may correspond with a higher milk price in some milk market areas of the country where farmers are paid more for the additional protein in the milk. The potential for greater profit motivated several studies examining the effect of niacin and protein on total milk and component yields.

In 1992, Lanham et al. (1992) expected to stimulate microbial protein synthesis and increase milk protein while investigating the effect of niacin (6 g/cow/d) and 0% or 15% whole cottonseed on 40 Holstein cows in mid to late lactation. While milk yields, milk composition, and glucose of cows supplemented with niacin alone were unaffected, diets containing both niacin and 15% whole cottonseed produced significantly higher milk yields and milk fat percentages. Authors suspected that the lack of treatment effect of niacin alone on milk protein

yield could be attributed to the cows being so late in lactation that ketosis was not present. Also, blood plasma revealed no elevation in NA with supplementation, indicating that niacin was not limiting or it was not absorbed. Since cows fed 6 g/cow/d niacin had the lowest dry matter intake (DMI), supplemental niacin in excess may have limited intake. However, feeding niacin along with cottonseed resulted in more milk and higher milk fat, perhaps due to the stimulatory properties of niacin on protein production in the presence of higher fat (Christenson et al., 1998).

Also in 1992, Zimmerman et al. (1992) reported DMI, milk yield, and milk component yield were unaffected by niacin supplementation (12 g/cow/d) along with either high or low dietary CP in 57 Holstein cows, 2 to 5 weeks post-partum. Researchers were hoping to take advantage of the property of niacin to reduce lipolysis, which often occurs when animals cannot acquire adequate energy from high protein diets. They theorized that niacin might increase protein synthesis and thus help alleviate milk fat depression. They observed that multiparous cows in early lactation experienced a tendency for lower BHBA levels, NEFA scores, and urine ketone levels while receiving high protein and niacin, indicating lower levels of lipolysis. It was proposed that there was a greater effect of niacin on multiparous cows and higher yielding cows under more stress because they are typically more susceptible to ketosis caused by an energy deficiency and subsequent increase in mobilization of fat stores (Zimmerman et al., 1992).

However, even among cows in which niacin appeared to lower the metabolic signs of ketosis, milk yields were not improved. There was no response to niacin in primiparous cows. Based on earlier research by Dennis et al. (1982), authors suggested that perhaps there was no treatment effect because the soybean meal was heat-treated, rendering niacin unavailable to rumen protozoa.

Three years later, Bernard et al. (1995) presented evidence in opposition to the idea that heat treated soybeans limited the bioavailability of niacin by feeding 6 g/cow/d niacin and either soybeans or heat-treated soybeans to 56 Jersey cows from 3 weeks prior to parturition until 15 weeks afterward. Whether niacin was supplemented along with heat-treated or non-heat-treated soybeans, there were no effects of treatment on DMI, milk production, or milk composition, and therefore it does not appear that heat-treatment of soybeans affects bioavailability of niacin.

Supplemental Niacin and Fat

The potential anti-lipolytic effect is another highly desirable characteristic of niacin supplementation. Cows in high production are often in negative energy balance, mobilizing extensive fat reserves that overwhelm the ability of the liver to metabolize the fat, resulting in excessive blood concentrations of NEFAs, release of BHBA, and onset of ketosis. Ketosis reduces total milk yield and component concentrations, and in order to compensate for the negative energy balance, cows

are often fed high-energy diets containing high concentrations of fat. Since cows are most seriously affected by ketosis during peak lactation around 30 to 40 days post-partum, effects on fat mobilization are most effectively observed during that time period.

Skaar et al. (1989) conducted a study in which niacin (12 g/cow/d) was supplemented to 39 Holstein cows from 17 days prior to parturition until 15 weeks in milk. Cows supplemented with niacin experienced less weight loss between weeks 2 and 4, and although insignificant, numerical milk yields of cows supplemented with niacin peaked lower than control cows on the study. There were no treatment effects on milk yield, milk fat or protein percentages, or plasma BHBA.

Madison-Anderson et al. (1997) studied the effect of supplemental unsaturated fat and niacin (12 g/cow/d NA) on 16 lactating Holstein cows for 4 weeks beginning at 53 ± 20 DIM. No treatment effects were observed on dry matter intake (DMI), body weight (BW), or energy-corrected milk (ECM). Milk components were not affected by niacin, although there was a significant interaction between fat and niacin that decreased milk fat and protein percentages when both were supplemented, and increased percentages when only niacin was supplemented. It should be noted that this study was conducted on cows past peak lactation, which may account for the lack of treatment effect. This result contrasted with that of Lanham et al. (1992) where supplemental niacin increased

milk fat percentage in late lactation cows also consuming cottonseed, but no effect was observed without cottonseed in the diet.

In 1998, two concurrent studies were conducted on 48 multiparous Holstein cows from 4 to 43 weeks in milk, supplemented with niacin (12 g/cow/d NA), liquid fat, or both. Diets were formulated devoid of supplemental rumen undegradable protein in order to maximize the opportunity for niacin to enhance microbial protein synthesis. Drackley et al. (1998) examined treatment effects on milk CP, milk yield, energy balance, and body condition score (BCS), while Grum et al. (2002) studied treatment effects on hepatic β-oxidation of palmitate and hepatic concentrations of total lipid, triglyceride, and glycogen.

Drackley et al. (1998) found that niacin (12 g/cow/d NA) alone increased milk yield 7.2% and improved 3.5% fat-corrected milk (FCM) and CP percentage. Yields of fat, CP, and true protein in milk tended to increase with niacin supplementation. There were no significant treatment effects on DMI, milk non-protein nitrogen (NPN), plasma NEFA, or plasma BHBA. It was apparent that cows supplemented with niacin stayed in negative energy balance longer than other cows on the trial, which disagrees with the findings of Skaar et al. (1989). Like Madison-Anderson et al. (1997), Drackley et al. (1998) did not expect to see a response to niacin supplementation on plasma BHBA levels because treatments were introduced after peak lactation when the cows were most susceptible to ketosis.

Grum et al. (2002) found a significant 3-way interaction between fat, niacin (12 g/cow/d NA), and week for total lipid and triglyceride in the liver, suggesting that niacin supplementation decreased lipolysis over time from 4 to 43 weeks in milk. Likewise, Skaar et al. (1989) found that niacin (12 g/cow/d NA) increased total concentrations of lipid and triglyceride in the liver earlier in lactation. The study by Grum et al. (2002) indicated that there was a tendency for lower glycogen concentration and suggested that this, in conjunction with the stable DMI demonstrated by Drackley et al. (1998), might indicate a greater glucose demand for milk synthesis with niacin supplementation. Niacin did not affect hepatic capacities for total or peroxisomal β-oxidation, despite being a precursor to NAD, which is a cofactor in β-oxidation.

In yet another attempt to demonstrate the anti-lipolytic properties of niacin and fat, Christensen et al. (1996) investigated the effect of supplemental niacin (12 g/cow/d NA) with or without supplemental fat, and studied the effect on nutrient flows to the small intestine, concentrations of plasma metabolites, and production of milk and milk components. Four multiparous Holstein cows were surgically fitted with ruminal and duodenal cannulae at around 30 DIM, and then subjected to each of four 21-day periods, fed dietary treatments consisting of low fat, low fat plus niacin (12 g/cow/d NA), high fat, and high fat plus niacin (12 g/cow/d NA). Supplementation of niacin tended to decrease DMI, which in turn decreased intake of OM, starch, and total amino acids, but not digestibility. There were no treatment

effects on concentrations of NEFA and BHBA in plasma, indicating no effect on lipolysis, yet as in many of the other niacin studies, this may have been due to cows being past peak lactation and no longer mobilizing fat stores. In this study, high versus low fat supplementation did not alter ruminal fermentation, microbial protein synthesis, or milk production and composition, and therefore associated responses to niacin could not be observed.

Two years later, Christensen et al. (1998), reported additional data from the same study reiterating that niacin (12 g/cow/d NA) supplementation did not significantly alter gross energy digestibility, flows of C_{18} fatty acids, total fatty acids, or individual long-chain fatty acids that affected milk fat production. There was a tendency for an interaction between niacin and fat for increasing net flow of C_{18} fatty acids in the high fat diets and reducing net flow in the low fat diets, but authors attribute this to the effect of dietary fat on ruminal fermentation, not niacin. Fatty acid composition of ruminal bacteria was not altered by niacin supplementation, but the $C_{18:0}$ concentration decreased when niacin was supplemented in the cows consuming the low fat diet and vice versa in cows receiving the high fat diets such that there was a significant interaction between fat and niacin. There was also a significant interaction between fat and niacin supplementation that decreased passage of $C_{16:0}$, $C_{18:0}$, total C_{18} fatty acids and total fatty acids to the duodenum with niacin supplementation in the low fat diets and vice versa in the high fat diets. Authors were unable to explain any of the

interactions observed. Overall it was determined that niacin supplementation did not alter energy or fatty acid utilization by dairy cows.

In a more recent effort to characterize the anti-lipolytic effects of niacin, French (2004) supplemented a therapeutic dose of niacin (48 g/cow/d NA) to 14 multiparous Jersey cows beginning 30 days prior to parturition. Cows receiving supplemental niacin experienced lesser decline in DMI than control cows during the last week of gestation and DMI was greater for cows receiving supplemental niacin the day prior to parturition. Plasma NEFA were reduced for cows supplemented with niacin the day prior to and the day following parturition. Authors concluded that niacin reduced plasma NEFA by 65% at parturition and is intimately related to DMI depression surrounding parturition.

Two years later Chamberlain and French (2006) conducted a second transition period experiment on 27 multiparous Holstein cows and 27 multiparous Jersey cows treated with supplemental niacin (0, 49, or 98 mg NA per kg BW) from 30 days pre-partum to 21 days post-partum. Although there was no treatment effect on pre-partum DMI, post-partum DMI increased when cows were supplemented with 49 mg/kg niacin. There were no treatment effects on NEFA or BHBA.

In 2009, Morey et al. (2009) supplemented rumen-protected niacin (24 g/cow/d) to 9 primiparous and 13 multiparous cows from 21 days prior to calving until 21 days postpartum. They found that rumen-protected niacin treatment

significantly decreased DMI by about 4 kg/cow/d during the last 5 days prepartum, but there were no treatment effects on intake postpartum. Rumen-protected niacin significantly reduced plasma BHBA and contributed to a similar trend in NEFAs. There were no treatment effects observed for liver triglyceride concentration, BCS, BW, milk yield, or milk component production.

To further clarify the impact of niacin supplementation on lipolysis and DMI, Spivey et al. (2009) ruminally cannulated 6 Holstein steers and continually infused niacin (0, 8, and 16 g/cow/d NA) into the abomasum. Then, steers were challenged with a β-agonist, isoprenol to trigger lipolysis. Blood samples were collected prior to isoprenol challenge and 8 minutes afterward. Niacin (16 g/cow/d) inhibited the isoprenol stimulated increases in plasma NEFA concentrations, but at the expense of large reductions in feed intake. Lower levels of niacin (8 g/cow/d) had no effect. Authors concluded that the negative impact of therapeutic doses of niacin may not be worth its anti-lipolytic properties.

Effect of Niacin on Glucose and Insulin

While Zimmerman et al. (1992) reported a significantly higher blood glucose level in multiparous cows given supplemental protein and niacin (12 g/cow/d) in early lactation, other studies revealed no treatment effects of niacin on blood glucose (Drackley et al., 1998; Christensen et al., 1996; Chamberlain and French,

2006; Di Costanzo et al., 2007). In one study, insulin tended to be elevated in diets supplemented with niacin (Lanham et al., 1992), which supports the theory that NA increases milk yield by impairing glucose tolerance and therefore makes glucose more available to the mammary gland for milk synthesis (Drackley et al., 1998).

Effect of Niacin on Heat Stress Tolerance

As per cow milk production has increased over the years, so has metabolic heat production and sensitivity to heat stress resulting in an estimated $900 million in annual losses for the US dairy industry (Collier et al., 2005). Heat stress is detrimental to aspects of dairying including natural estrus cycles, embryo survival, DMI, milk production, and profitability. The magnitude of heat stress is affected by ambient temperature, humidity, solar radiation, and wind speed (Dikmen and Hansen, 2008).

There are several different methods and mechanisms available to quantify the level of heat stress affecting cattle, and there is some debate over which is most accurate. The temperature-humidity index (THI) is a calculated value representing the level of thermal stress on an animal caused by a combination of temperature and humidity (Bohmanova et al., 2006). There are many different THI equations available and in order to calculate a useful value, a researcher must objectively choose the most appropriate equation. Although it does not fully reflect

the rampant humidity of the southeast, the equation that most effectively predicts heat stress in cattle is THI = 0.81 × T + 0.143 × RH + 0.0099 × RH × T + 46.3, T = temperature, RH = relative humidity (Bohmanova et al., 2007). A THI ≥ 72 indicates heat stress, which usually represents a sharp decline in milk production (Bohmanova et al., 2007). West et al. (2003) demonstrated that milk yield and DMI are most closely associated with the THI and mean air temperatures 2 days prior to sampling. Daily highs had the greatest impact on evening milk yields and evening lows had the greatest impact on morning milk yields (West et al., 2003). There is also a black globe index thought to be more accurate than THI, which considers the impact of solar radiation in addition to temperature and humidity (Collier et al., 2005). Black Globe is not widely used because the technology to measure solar radiation is not present on most research farms.

Infrared thermography devices can be used in research studies to estimate the skin temperatures of animals; if the temperature is below 35°C, the animal is able to effectively dissipate heat (Collier et al., 2005). In 2001, Umphrey et al. (2001) published a study indicating that rectal temperature could be correlated to milk yield, milk fat percentage, milk fat yield, milk protein yield, DMI, and respiration rate, yet skin temperature was unrelated to all variables measured. Core body temperature can be monitored using a rectal or intravaginal probe that remains inside the animal for up to 6 days, recording core body temperature at regular intervals. It has also been shown that normal core temperatures among

cows in a thermoneutral environment are near 38.6°C, and milk production declined if exposed to ambient temperatures exceeding 39°C for more than 16 hours (Igono and Johnson, 1990).

Mechanisms for abating heat stress include fans to improve air movement, soaking cows' bodies with water, high pressure water mist, facilities to provide shade, and dietary manipulation. For our purposes here, we are going to focus specifically on dietary manipulation. Since heat stress causes DMI to decrease, diets must be formulated with increased nutrient density, altered mineral and water requirements, and altered digestive tract function (Collier et al., 2005). Additionally, it is believed that niacin supplementation may play a role in abating heat stress through its vasodilatory properties.

The first published study relating niacin supplementation to heat stress was conducted by Muller et al. (1986). Niacin (6 g/cow/d) was supplemented to 240 Holstein multiparous and primiparous cows from 5 herds in Pennsylvania averaging 143 DIM from July through September. It was found that cows receiving niacin increased milk yields and corresponding fat and protein yields, but not fat or protein percentages.

It was more than 10 years before another study was published analyzing the connection between niacin supplementation and heat stress tolerance. Di Costanzo et al. (1997) studied the effect of niacin supplementation on thermoregulation in 26 Holstein cows beginning at approximately 90 DIM under

heat stress conditions ranging between THI values of 63.1 and 85.1. Niacin supplementation among the cows receiving treatment increased in concentration over three consecutive 17-day periods from 12 g/cow/d to 24 g/cow/d to 36 g/cow/d NA. Treatments did not affect milk production, 4% FCM, BW, urea nitrogen, NEFA, or rectal temperatures. These results contradict the findings of Muller et al (1986) who observed increased milk and component yields while only supplementing a 6 g/cow/d of niacin.

Di Costanzo et al. (1997) also used an infrared thermometer to measure three daily skin temperatures from shaved areas on the tail and rump of each cow. It was determined that cows supplemented with niacin had lower skin temperatures on the rump at 0800 and 1600 hours during the first 17-day period, and the tail skin temperatures followed the same trend. Tail skin temperatures were also significantly lower at 0800 h during the second 17-day period followed by similar trend at 1600 and 2200 h. There were no differences from control during the third 17-day period. Rectal temperatures during all three periods remained constant and authors proposed two explanations for the reduced skin temperatures with constant rectal temperatures: (1) heat transfer was reduced so the cows gained less heat from the environment while maintaining a stable core body temperature, or (2) evaporative heat loss was increased while heat gain was regulated such that cows maintained a stable core body temperature. Yet, if skin

temperature is not an accurate gauge of heat stress, this data may not be useful (Umphrey et al., 2001).

Another 10 years passed before a third pair of heat stress studies was conducted, this time using encapsulated niacin to protect the niacin from rumen degradation in hopes of observing a more repeatable niacin effect on lactating cows suffering from heat stress. In 2007, Zimbelman et al. (2007) presented work supplementing rumen-protected niacin (12 g/cow/d Niashure™) to 12 multiparous Holstein cows exposed to thermoneutral or heat stress conditions. Cows receiving supplemental niacin experienced increased DMI, recorded lower average vaginal temperatures measured at 15-minute intervals, and increased sweating rate during peak thermal load between 1100 and 1600 hours. Earlier studies with niacin, not involving heat stress, cited lower DMI or no change in DMI (Di Costanzo et al., 1997; Muller et al., 1986). Skin temperatures were not affected by treatment. This work completely contradicts the results of Di Costanzo et al. (1997) that indicated niacin supplementation reduced skin temperatures but not rectal core temperatures. However, this may also validate earlier findings suggesting that skin temperature is not a reliable indicator of heat stress effects (Umphrey et al., 2001).

Two years later, Zimbleman et al. (2008) followed up with a second study indicating that rumen-protected niacin (12 g/cow/d Niashure™) significantly reduced the effects of summer heat stress in 400 lactating primiparous and multiparous Holstein cows averaging 166 DIM in the hot, dry conditions of Arizona.

Rumen-protected niacin supplementation increased fat and protein concentrations, and subsequently FCM and ECM yields. Niacin supplementation also decreased vaginal core body temperatures during peak thermal load from 1300 to 1600 hours in a subset of 16 cows. There were no effects on milk yield or DMI, which disagrees with Muller et al. (1986) who found that niacin increased milk yield, but agrees with Di Costanzo et al. (1997).

Summary

Traditionally, niacin was considered a non-essential vitamin because it is synthesized by rumen flora in great enough quantities to fulfill the needs of the moderately productive cow. Today, the modern dairy industry has enhanced the productive capacities of the cow, and simultaneously increased nutritive needs and production of heat that must be dissipated. Supplemental niacin may be advantageous in meeting the enhanced nutritive needs of the cow in order to maximize her productive capacity without provoking disease onset, and may also play a role in aiding the cow in dissipation of heat. The most recent studies have almost exclusively focused on the ability of rumen-protected niacin to improve parameters of production efficiency and health under heat stress conditions.

In dairy cows, niacin is naturally synthesized by microbes in the rumen, and is also available in grains and supplements. There are two known forms of niacin

distinguished by their side groups: nicotinamide (NAM) and nicotinic acid (NA). Once ingested, it appears that most NAM is quickly hydrolyzed to NA in the rumen and abomasum, which is immediately utilized or degraded by rumen bacteria and protozoa. It has been proposed that niacin enhances microbial protein synthesis directly, or it enhances protozoal populations that maintain rumen environments favorable for natural rumen flora. Regardless, very little exogenous niacin reaches the duodenum where it can be absorbed, and ultimately, the cow acquires her niacin by liberating it from within digested rumen bacteria.

Since niacin supplementation has been shown to increase microbial protein synthesis and to have anti-lipolytic effects, it was proposed as a means to improve milk fat and protein yields by decreasing ketosis incidence and rendering protein available for incorporation into milk. Studies have failed to demonstrate that niacin supplementation increases milk protein percentage yields, even when supplemental protein was provided with the niacin. There were several studies that indicating that niacin reduced BHBA and NEFA levels associated with ketosis during peak lactation, but most studies were conducted beyond peak lactation when the cows were less susceptible to metabolic disease as a result of fat mobilization. Increased milk fat yields associated with niacin supplementation were inconsistent, but any effect of niacin appeared to be associated with the feeding of supplemental fat.

The final property of niacin investigated by researchers is its ability to maintain production levels in dairy cows under extreme heat stress conditions. While earlier studies suggested niacin supplementation enhanced milk and protein yields, and lowered skin temperatures in high heat, general heat stress studies suggested that skin temperatures were not a reliable indicator of heat stress or heat dissipation. More recent studies supplemented rumen-protected niacin and indicated that it decreased core body temperature and improved ECM and FCM yields under heat stress conditions.

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RUNNING HEAD: DAIRY, FEED EFFICIENCY, HEAT STRESS, NIACIN

Effect of Feeding Supplemental Rumen-Protected Niacin (Niashure™) on Milk Yield and Milk Composition in Early Lactation Holstein Cows

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ABSTRACT

Eighty-six multiparous Holstein cows were assigned to one of three treatments to investigate the effects of 0, 6 and 12 g/d supplemental rumen-protected niacin (Niashure™) from 35 to 142 days in milk (DIM). Cows received a corn silage based total mixed ration fed *ad libitum* twice daily through individual Calan® feeding stations. At the morning feeding all cows received 21 g/d of a topdress composed of a bentonite carrier blended with treatments of 0, 6, or 12 g/d Niashure™. Feed intakes were recorded daily. Analysis of weekly feed samples composited monthly indicated that diet dry matter (DM) contained 18.3% crude protein (CP) and 21.5% acid detergent fiber (ADF). Milk yields were recorded at each milking (2x/d). Weekly milk samples composited from consecutive morning and afternoon milkings were analyzed for fat, protein, urea nitrogen, and lactose. High and low ambient temperatures and humidity levels in the free-stall barn were recorded daily. Skin temperatures were measured at the rear udder attachment five days per week at the p.m. milking using an infrared thermometer. Body weights and body condition scores were measured weekly. Data were analyzed using the mixed procedure of SAS® and significance was declared at $P < 0.05$. There were no significant treatment effects on skin temperatures, body weights, or body condition scores. Milk yield (kg/d), fat (%), protein (%), lactose (%), milk urea nitrogen (MUN) (mg/dL), and dry matter intake (DMI) (kg/d) were (38.4, 4.0, 2.9, 4.8, 21.1, 24.9), (40.2, 3.9, 2.8, 4.7, 21.3, 24.9),

and (40.0, 4.0, 2.9, 4.8, 21.3, 24.5), for treatments of 0, 6, and 12 g/d Niashure™, respectively, with no significant treatment effects. In comparison to control, Niashure™ supplementation of 12 g/d tended to improve feed efficiency (kg milk / kg DMI) from 1.58 to 1.65 ($P < 0.08$). In comparison to control, supplementation of 6 g/d Niashure™ significantly improved feed efficiency from 1.58 to 1.69 ($P < 0.02$).

(**Key words:** dairy, feed efficiency, heat stress, niacin)

Abbreviation Key: **ADF** = acid detergent fiber; **BHBA** = beta hydroxybutyrate; **CP** = crude protein; **DIM** = days in milk; **DM** = dry matter; **DMI** = dry matter intake; **ECM** = energy corrected milk; **EDTA** = ethylenediaminetetraacetic acid; **FCM** = fat corrected milk; **ME** = mature equivalent; **MUN** = milk urea nitrogen; **NEFA** = non-esterified fatty acids; **NRC** = National Research Council; **SNF** = non-fat solids; **THI** = temperature-humidity index; **TMR** = total mixed ration.

INTRODUCTION

In 1978, the National Research Council (1978) stated that niacin synthesized naturally by microbes inside the rumen was adequate for optimal dairy performance. In the 30 years since then, the average annual milk yield (kg/cow/yr) in the United States has increased by approximately 60% from 5,100 to 8,599 in 2004 (USDA, 2005). It is widely speculated that the niacin requirement to support this enhanced level of production in dairy cows today is above that which can be produced naturally by the dairy cow, and that supplementation of niacin may increase her production potential. Nevertheless, efforts to supplement niacin have shown inconsistent results in terms of improved milk yield, milk components, blood non-esterified fatty acids (NEFA) and beta-hydroxybutyrate (BHBA), and heat stress tolerance. This inconsistency in results may be due to the degradation of niacin by rumen microbes following ingestion (Santchi et al., 2004; Schwab et al.,

2006). Recently, technology has become available that enables supplemental niacin to resist ruminal degradation and be absorbed in a biologically active form in the intestine. One rumen-protected niacin product available is Niashure™ (Balchem, Inc., New Hampton, NY), which is protected by a patented lipid capsule. Two studies conducted in the hot, dry summer conditions of Arizona with multiparous and primiparous cows have indicated that Niashure™ reduces core body temperature while improving fat-corrected milk (FCM) and energy-corrected milk (ECM) yields (Zimbelman et al., 2007; Zimbleman et al., 2008).

The objectives of this trial were to determine the effects of feeding 0, 6, and 12 grams per day of a rumen-protected niacin supplement (Niashure™) to early lactation multiparous Holstein cows on milk yield, milk composition, NEFAs, BHBA, and skin temperatures.

MATERIALS AND METHODS

Following parturition, 92 multiparous Holstein cows from the North Carolina Department of Agriculture Piedmont Research Station – Dairy Unit (Salisbury, NC) were blocked by previous lactation ME milk yields and randomly assigned to one of three treatment groups. Six cows were removed from the trial due to health problems unrelated to treatments. Rumen-protected niacin (Niashure™, Balchem Corporation, New Hampton, NY) was blended with sodium bentonite as a carrier to provide 0, 6, or 12 g Niashure™ daily in a 21-g dose of supplement.

Supplements were top-dressed on the morning feeding inside each cow's individual Calan® feeding station (American Calan Inc., Northwood, NH). Cows were trained to use feeding stations inside a free-stall barn immediately following parturition and received their assigned dietary treatments for approximately 115 days ranging from about 15 to 161 days in milk (DIM). All cows received the same corn silage-based total mixed ration (TMR), formulated in accordance with National Research Council (NRC) nutritional standards for the lactating dairy cow producing 38.6 kg milk daily. Cows were fed a TMR twice daily for *ad libitum* intake and 10% orts. Feed intakes based on orts and feed allocations were measured daily using a Data Ranger® computerized, self-propelled vehicle (American Calan Inc., Northwood, NH).

Sample Collection

Total mixed ration samples were collected weekly and frozen at -17ºC. Samples were thawed and dried for 48 hours in a 60°C oven and ground through a Wiley Mill® (Thomas Scientific, Swedesboro, NJ) fitted with a 1 mm screen. They were composited by month and analyzed for dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), protein fractions, and minerals at Cumberland Valley Analytical Services (Hagerstown, MD). Since there was little variation in the monthly TMR analyses, an additional 12-month

composite was submitted to Cumberland Valley Analytical Services for a more comprehensive analysis of protein, fat, and fiber components in the diet.

Cows were milked twice daily, and milk yields were automatically recorded by AFIFarm farm management software (S.A.E. Afikim, Kibbutz Afikim, Israel). Weekly milk samples from each cow were composited from consecutive morning and evening milkings and analyzed by the United DHIA Laboratories (Blacksburg, VA) for fat, protein, urea nitrogen, non-fat solids (SNFs), and lactose composition. Fat, protein, and urea nitrogen were determined by infrared spectrophotometry (Bentley ChemSpec 150 Analyzer, Chaska, MN) and SNF levels were derived by a mathematical equation (Tyrrell and Reid, 1965). Urea nitrogen was measured using a modified Berthelot reaction (Chaney and Marbach, 1962). Skin temperatures were detected with an infrared thermometer (Extech® Instruments Corporation, Waltham, MA) in a hairless area near the rear udder attachment 5 days per week during the evening milking. Ambient high and low temperatures and humidity levels were also recorded for each 24-hour period during the trial.

Blood samples were collected prior to the morning feeding on days 35 ± 3, 42 ± 3, 49 ± 3, and 56 ± 3. Blood was collected from a caudal vessel into two 10-mL Monoject© vacuum tubes (Covidien Ltd., Mansfield, MA) and chilled on ice during the collection process; one tube contained ethylenediaminetetraacetic acid (EDTA) to prevent clotting and the other contained no additive. The EDTA tubes

were spun in a centrifuge at 20,000 x g for 20 minutes. Plasma supernatant was decanted and frozen at -17ºC in non-sterile polypropylene tubes (Fisherbrand, Hanover Park, IL). The second vacuum tube was refrigerated for 1 to 2 hours, decanted, and frozen likewise to the plasma. Frozen plasma was sent to Michigan State University Diagnostics Laboratories (Lansing, MI) for analysis of non-esterified fatty acids (NEFA) and beta hydroxybutyrate (BHBA) concentrations. A Wako Diagnostics (Richmond, VA) kit was used to measure NEFAs and a Catachem (Bridgeport, CT) kit was used to measure BHBA; all samples were run on an Olympus AU 800 chemistry analyzer (Olympus America Inc., Center Valley, PA).

Body weights (BWs) and body condition scores (BCS; 1 to 5 scale) (Ferguson et al., 1994) were recorded once weekly for each cow during the trial.

Statistical Analysis

Following parturition, 92 multiparous Holstein cows were blocked by calving dates and previous lactation ME milk yields, and assigned to dietary treatments. Data from 86 cows were analyzed by repeated measures ANOVA (Littell et al., 1998) using the mixed procedure with autoregressive (1) covariance structure (SAS® Institute, 2004). Least squares means for treatments were compared using

the PDIFF option to carry out the least significant difference procedure with statistical significance declared at $P < 0.05$.

RESULTS AND DISCUSSION

Feed Efficiency

Niashure™ supplementation improved FE when calculated as milk yield/DMI ($P < 0.05$), as a result of the numerical increase in milk yield and the numerical decrease in dry matter intake (DMI) with increasing Niashure™ dose. Feed efficiency values (milk yield/DMI) resulting from supplementation of 0, 6, and 12 g Niashure™ were 1.58, 1.69, and 1.66, respectively. Other measures of feed efficiency, ECM/DMI and FCM/DMI, were not significantly affected by treatment, but a numerical increase was observed with increasing supplemental Niashure™ (Table 3).

Feed Intake, BW, and BCS

There were no treatment effects on DMI, BW, BW gain, or BCS (Table 3). Likewise, Zimbleman et al. (2008) found no treatment effect of Niashure™ on DMI, but Zimbelman (2007) recorded an increase in DMI under heat stress conditions.

Trials that supplemented un-protected niacin are numerous, but provide widely inconsistent results.

Milk Yield and Composition

There were no significant treatment effects on milk yield or milk component concentration or yields. Because the mean milk fat percentage for all treatments was 3.96 ± 0.09, 4% ECM and 4% FCM were calculated and no significant treatment effects were observed. However, there were numerical increases in milk yield, 4% ECM, and 4% FCM, which contributed to the significant improvement in FE (milk yield/DMI) (Table 3). These numerical trends in milk yield suggest that with additional cows added to the study, a significant change in milk yield may have been observed.

Zimbleman et al. (2008) also found that 12 g/d Niashure™ did not increase milk yield, but did increase both fat and protein concentrations, and ECM and FCM yields. Previous studies using un-protected niacin are numerous, but effects on milk yields and composition are inconsistent.

Blood Metabolites

There were no treatment effects on NEFA or BHBA concentrations in blood plasma (Table 3). Cows began receiving treatments at approximately 26 DIM, such that the experiment may have begun too late in lactation to detect effects on plasma NEFA and BHBA concentrations. Campbell et al. (1994) observed similar results after initiating 6 to 12 g/cow/d un-protected niacin supplementation at 200 DIM, and suggested that their study was conducted too late in lactation for results to be pertinent to the potential anti-lipolytic properties of niacin. Dufva et al. (1983) observed a decrease in NEFAs and BHBA immediately following parturition when they began feeding cows 6 g/cow/d un-protected niacin 2 weeks pre-partum, increased niacin supplementation to 12 g/cow/d immediately post-partum, and predisposed cows to ketosis by feeding nutrient-rich diets prior to calving. Abomasal infusion of 6 to 60 g un-protected niacin in feed-restricted cows caused a decrease in NEFA levels followed by a dramatic rebound above starting levels before stabilizing, indicating a transient inhibition of lipolysis (Pires and Grummer, 2007). However, even while feeding 12 g/cow/d unprotected niacin beginning 19 days prepartum, Minor et al. (1998) observed no effect of niacin on NEFA and BHBA levels.

Skin Temperatures

There were no effects of niacin treatments on skin temperature, although skin temperature was affected by weeks in milk ($P < 0.01$). There was a trend for supplemental niacin to affect skin temperature when difference from average control temperature was calculated to eliminate the impact of ambient temperature changes ($P < 0.10$), however, differences were very low. It cannot be explained why 6 g Niashure™ tended to increase skin temperature (+0.07°C) and 12 g Niashure™ tended to lower it (-0.05°C). Note that only about 9% of skin temperature values were collected during June, July, and August, when the effects of summer heat stress are normally observed on dairy production levels in the southeastern United States (Bohmanova et al., 2006). The remaining skin temperature values were collected throughout the remainder of the year, when ambient temperatures and humidity levels are not expected to alter production levels in dairy cattle.

Researchers in Tucson, Arizona, found that 12 g/cow/d Niashure™ reduced vaginal core temperatures during daily periods of peak ambient temperatures ($P < 0.01$) (Zimbleman et al., 2008). It is important to note that this trial was conducted in Arizona where the climate is much hotter and drier than in North Carolina where the current trial was held. It is unknown how core temperatures relate to skin temperatures, but one study indicated that skin temperatures are unrelated to all other measures of production and heat stress, including core body temperature

(Umphrey et al., 2001). We propose that elevated skin temperatures may indicate an increased loss of body heat, resulting in reduced core body temperature.

Di Costanzo et al. (1997) found that 12 to 36 g/cow/d un-protected niacin supplemented to cows under heat stress conditions tended to decrease skin temperatures at the tail and rump ($P < 0.10$), but did not affect rectal core temperatures. Two theories were proposed explaining why skin temperature decreased but core temperature remained consistent: (1) Niacin reduced heat transfer so ambient heat was not gained and core temperature was maintained; and (2) Niacin increased evaporative heat loss so the skin temperature was lower than the core temperature (Di Costanzo et al., 1997).

As expected in North Carolina, ambient temperatures inside the free stall barn where cows were housed fell to a nadir from November to February at about 4.4°C, began to climb in March, and peaked at over 37.8°C from June through September before declining again in October. No relationships were observed between ambient temperatures or humidity levels and production parameters measured in this trial. The temperature humidity index (THI) could not be accurately calculated as an indicator of heat stress because the temperatures and humidity levels recorded were the highs for each 24-hour period, not necessarily highs corresponding to a single point in time; humidity likely peaked in the early morning whereas temperature most likely peaked in the mid-afternoon, consequently exaggerating the THI.

CONCLUSIONS

Feed efficiency expressed as milk yield/DMI (kg/kg) was significantly improved ($P < 0.05$) by 6 and 12 g supplemental Niashure™ in fresh multiparous Holstein cows. There was also a tendency for improved FE ($P = 0.13$) when milk yield was expressed as FCM or ECM. There was a trend for treatment effect on skin temperature relative to control ($P < 0.1$), but it is unexplained why 6 g Niashure™ increased skin temperature and 12 g decreased it. It is suggested that increases in skin temperature indicate loss of body heat and reduction of core body temperatures. Niashure™ supplementation did not significantly affect milk yields or DMI, although numerical improvements were observed.

ACKNOWLEDGEMENTS

The authors thank Correll Hall and the staff at the Piedmont Research Station for their hard work and attention to detail through out the entire duration of this trial. Appreciation is also extended to Dr. Edgar Franco Abad for his guidance in the laboratory, and his assistance with the blood and feed sample analyses.

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TABLE 1. **Ingredient composition of dietary treatments (% of DM)**

Ingredient	Basal TMR, % of DM
Corn silage, unprocessed	41.0
Whole cottonseed	13.1
Alfalfa hay	9.3
Corn grain, ground	10.6
48% soybean meal	13.5
Corn gluten feed	9.4
Dicalcium phosphate	0.05
Calcitic limestone	1.5
Salt, plain white	0.37
Dynamate®[1]	0.20
Vitamax Plus 2X Premix[2]	0.09
Sodium bicarbonate	0.83
Geobond®[3]	0.07

[1]22% S, 18% K, and 11% Mg (The Mosaic Company, Plymouth, MN).

[2]8.9% Ca, 0.4% P, 0.1% Mg, 0.3% K, 1% S, 0.1% Cl, 10,572 mg/kg Fe, 18,432 mg/kg Zn, 4,380 mg/kg Cu, 12,504 mg/kg Mn, 101 mg/kg Se, 334 mg/kg Co, 359 mg/kg I, 1,200,830 IU/kg vitamin A, 300,775 IU/kg vitamin D_3, and 7,000 IU/kg vitamin E (Renaissance Nutrition, Inc., Roaring Spring, PA)

[3]70% SiO_2, 10% Al_2O_3, 4% Fe_2O_3, 2% K_2O, 1.5% MgO, 1% CaO, 11.5% Na, P, S, and L.O.I. (Bennett Mineral Company, Walkerton, VA).

TABLE 2. **Chemical composition of dietary treatments** [1,2]

Item[3]	Mean
DM, % of diet	63.0
CP	18.3
NDF	33.4
ADF	21.6
NFC[4]	37.7
Ash	6.70
Ca	0.80
P	0.47
Mg	0.27
K	1.51

[1]Cumberland Valley Analytical Services, Hagerstown, MD

[2]All means are reported as a percentage of DM unless otherwise indicated.

[3]Analysis conducted with TMR samples (n=90) composited into monthly samples.

[4]NFC = 100 – (NDF + CP + fat + ash).

TABLE 3. **Daily milk yield, milk composition, intake, BW, and BCS as affected by dietary treatment.**[1]

Item	Dietary Treatments			SEM	*P* ≤
	Control	6 g Niashure™[2]	12 g Niashure™[2]		
Milk					
Yield, kg/d	38.4	40.2	40.0	0.8	0.25
True protein, %	2.86	2.80	2.85	0.03	0.39
True protein, kg/d	1.10	1.13	1.14	0.02	0.35
Fat, %	3.99	3.88	4.02	0.09	0.42
Fat, kg/d	1.53	1.55	1.62	0.05	0.38
MUN, mg/dL	21.05	21.33	21.30	0.40	0.87
Lactose, %	4.78	4.74	4.78	0.04	0.71
DMI, kg/d	24.3	23.8	23.7	0.5	0.67
4% ECM[3], kg/d	36.8	37.8	38.9	0.9	0.30
4% FCM[4], kg/d	38.3	39.4	40.4	0.9	0.28
ECM/DMI, kg/kg	1.50	1.56	1.59	0.03	0.13
FCM/DMI, kg/kg	1.56	1.63	1.66	0.03	0.13
Milk yield/DMI, kg/kg	1.58	1.69	1.66	0.03	0.04
BW, kg	622	601	602	10	0.23
Gain, kg	47	47	52	7	0.81
BCS[5]	2.23	2.31	2.27	0.04	0.29
NEFA, mEq/L	0.43	0.42	0.45	0.04	0.67
BHBA, µmol/L	405	381	402	14	0.28
Skin temperature, °C	34.6	34.2	34.4	0.3	0.78
Difference from control[6],°C	0.04	-0.07	0.05	0.04	0.07

[1]Data represents means from the duration of the trial.

[2]Fatty acid encapsulated niacin supplement (Balchem Inc., New Hampton, NY)

[3]4% ECM (kg/d) = milk yield (kg/d) × [(0.0929 × % fat) + (0.0563 × % true protein) + (0.0395 × % lactose)] divided by 0.749 NE_L (Mcal/kg) (NRC, 2001).

[4]4% FCM (kg/d) = [milk yield (kg/d) × 0.4] + [15 × (% fat / 100) × milk fat (kg/d)] (NRC, 2001).

[5]5-point scale where 1 = very thin to 5 = obese (Ferguson et al., 1994).

[6] Average control skin temperature for each day minus average skin temperature of cows on each treatment each day.

APPENDICES

APPENDIX 1

Average Milk Yield per Cow and Number of Cows on Trial

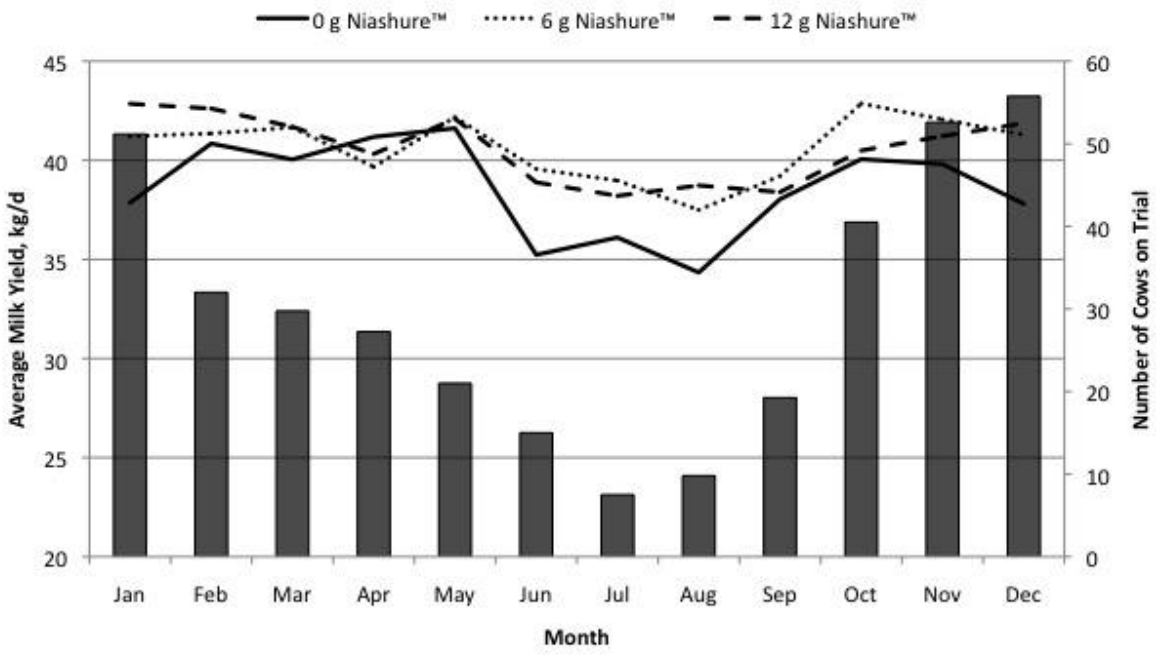

Figure 1. Average daily milk yields of cows receiving 0, 6, or 12 g Niashure™ by month and average total number of cows on trial each month.

Table 1. **Average daily milk yields of cows receiving 0, 6, or 12 g Niashure™ by month and average total number of cows on trial each month.**

Month	Control	6 g Niashure™	12 g Niashure™	Total Cows on trial
Oct 07	42.5	43.2	38.5	11
Nov 07	42.4	41.7	38.8	23
Dec 07	41.9	41.7	40.5	31
Jan 08	41.5	41.9	41.4	35
Feb 08	40.8	41.4	42.6	32
Mar 08	40.0	41.7	41.7	30
Apr 08	41.2	39.7	40.3	27
May 08	41.6	42.2	42.1	21
Jun 08	35.2	39.6	38.9	15
Jul 08	36.1	39.0	38.2	8
Aug 08	34.3	37.5	38.7	10
Sep 08	38.0	39.2	38.4	19
Oct 08	37.6	42.5	42.5	30
Nov 08	37.2	42.4	43.6	30
Dec 08	33.7	40.9	43.2	25
Jan 09	34.2	40.5	44.3	17

APPENDIX 2

Average Daily Ambient Temperatures

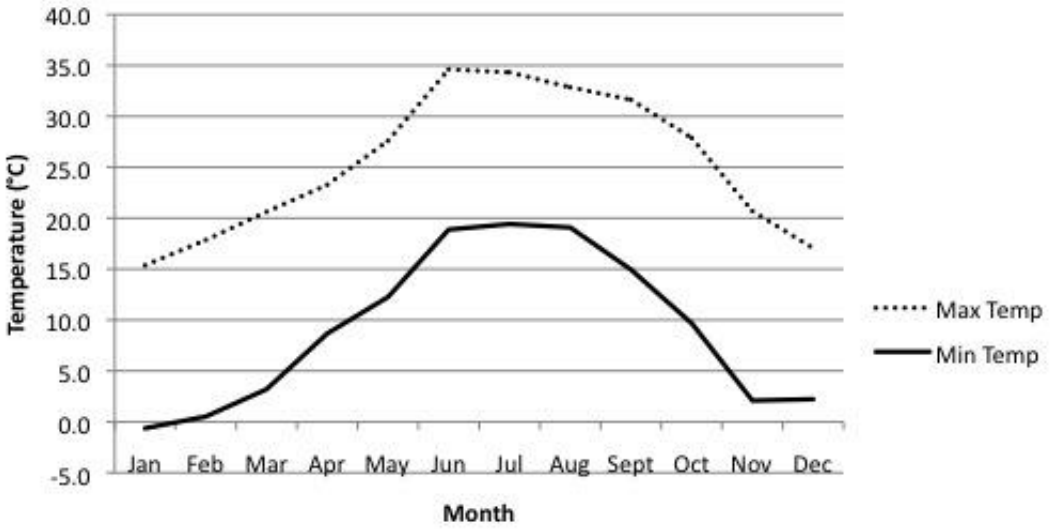

Figure 2. Average daily high and low ambient temperatures within the freestall barn, averaged by month.

Table 2. **Average daily high and low ambient temperatures within the freestall barn each month.**

Month	Max Temp, °C	Min Temp, °C
Oct 07	28.8	11.6
Nov 07	21.4	1.7
Dec 07	17.5	1.2
Jan 08	15.2	-1.4
Feb 08	17.4	2.0
Mar 08	20.6	3.2
Apr 08	23.3	8.7
May 08	27.6	12.3
Jun 08	34.6	18.9
Jul 08	34.3	19.4
Aug 08	32.9	19.1
Sept 08	30.1	17.2
Oct 08	27.0	7.8
Nov 08	20.0	2.4
Dec 08	16.6	3.2
Jan 09	15.5	0.1

APPENDIX 3

Average Daily Feed Intakes, Milk Yields, and High and Low Ambient Temperatures

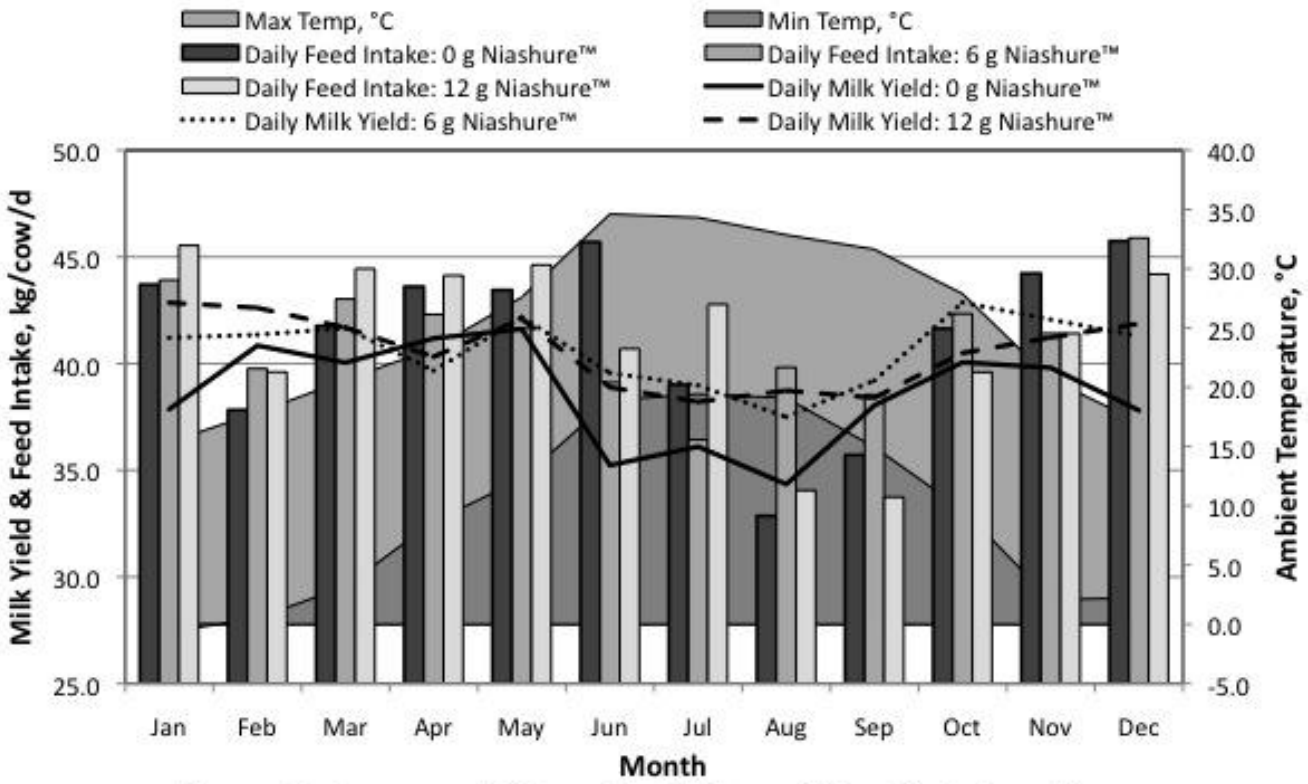

Figure 3. Average daily milk yields and feed intakes of cows receiving 0, 6, or 12 g Niahsure™ by month, and average daily high and low ambient temperatures inside the freestall barn each month.

Table 3. **Average daily milk yields and feed intakes of cows receiving 0, 6, or 12 g Niashure™ by month, and average daily high and low ambient temperatures each month.**

Month	Average Daily Milk Yield, kg/cow/d			Average Daily Feed Intake, kg/cow/d			Ambient Temperature, °C	
	0 g Niashure™	6 g Niashure™	12 g Niashure™	0 g Niashure™	6 g Niashure™	12 g Niashure™	Max Temp	Min Temp
Oct 07	42.5	43.2	38.5	41.5	42.1	39.4	28.8	11.6
Nov 07	42.4	41.7	38.8	42.8	41.4	39.6	21.4	1.7
Dec 07	41.9	41.7	40.5	45.1	46.4	42.5	17.5	1.2
Jan 08	41.5	41.9	41.4	44.8	43.6	44.2	15.2	-1.4
Feb 08	40.8	41.4	42.6	37.8	39.8	39.6	17.4	2.0
Mar 08	40.0	41.7	41.7	41.8	43.0	44.4	20.6	3.2
Apr 08	41.2	39.7	40.3	43.6	42.3	44.1	23.3	8.7
May 08	41.6	42.2	42.1	43.4	41.6	44.6	27.6	12.3
Jun 08	35.2	39.6	38.9	45.7	39.1	40.7	34.6	18.9
Jul 08	36.1	39.0	38.2	39.0	36.4	42.8	34.3	19.4
Aug 08	34.3	37.5	38.7	32.9	39.8	34.1	32.9	19.1
Sep 08	38.0	39.2	38.4	33.9	36.8	31.9	30.1	17.2
Oct 08	37.6	42.5	42.5	41.8	42.6	39.8	27.0	7.8
Nov 08	37.2	42.4	43.6	45.7	41.4	43.2	20.0	2.4
Dec 08	33.7	40.9	43.2	46.4	45.3	45.9	16.6	3.2
Jan 09	34.2	40.5	44.3	42.6	44.2	46.9	15.5	0.1