EFFECTS OF SUPPLEMENTAL LYSINE ON PERFORMANCE, ANTIBODY TITER AND RECTAL TEMPERATURE IN RESPONSE TO A MODIFIED-LIVE VIRAL VACCINE IN NEONATAL CALVES

[1]Kate Sharon, [1]Glenn Duff, [3]Jeff Dailey, [3]Jeff Carroll, [2]Jonathan Hilmer and [2]Brian Bothner

[1]Department of Animal and Range Sciences,
[2]Department of Chemistry and Biochemistry,
Montana State University, Bozeman, Montana, USA
[3]Livestock Issues Research Unit, USDA-ARS, Lubbock, Texas, USA

ABSTRACT

Infectious Bovine Rhinotracheitis (IBR), caused by bovine herpesvirus-1, contributes significantly to economic losses in the dairy and beef cattle industry. Lysine has been shown reduce virulence of herpesviruses in felids and humans. Our objective was to evaluate the effects of supplemental lysine on serum IBR antibody titer and rectal temperature in response to a modified-live Intranasal (IN) or Intramuscular (IM) respiratory-virus vaccination. Sixty-four neonatal Holstein bull calves (7 ± 2 d of age; BW = 37 ± 4.2 kg) were used in a completely randomized design. Calves were fed milk replacer supplemented with either 17 g/d L-lysine monohydrochloride (LYS; 28 calves) or an equivalent amount of casein (CAS; 28 calves) for 42 d. Calves were then vaccinated with either an IN IBR-Parainfluenza virus-3 (PI3) or an IM (IBR-PI3-bovine viral diarrhea type I and II, bovine respiratory syncytial virus) modified-live vaccine on d 36. A control group (8 calves) received no supplement or vaccination. All calves were housed in individual calf pens (1.2×2.1 m). Daily feed intakes were monitored and BW measured weekly. Calves were bled on d 0, 35, 36, 37 and 42. Temperature data loggers were attached to rectal probes and temperatures were recorded every 5 min from d 28 to d 42. No significant differences were determined for average performance, rectal temperature, or IBR antibody titers with either IN or IM vaccinations between LYS and CAS treated calves ($p>0.10$). However, serum urea nitrogen and the ratio of serum lysine: Arginine increased ($p<0.05$) for LYS compared to CAS calves. These results suggest that supplementing lysine impacts nitrogen metabolism but does not alter the response to IBR vaccination or animal performance in neonatal Holstein calves.

Keywords: Holstein Calves, Bovine Herpesvirus-1, Lysine, Vaccination

1. INTRODUCTION

The susceptibility of the respiratory tract to bacterial disease following a viral infection is a matter unresolved. Bovine Herpesvirus-1 (BHV-1) is one of the foremost infections contributing to Bovine Respiratory Disease (BRD; Nandi *et al.*, 2009). An infection of BHV-1 can lead to Infectious Bovine Rhinotracheitis (IBR), suppressing the immune system and increasing the risk fora secondary bacterial infection (Yates, 1982). Herpes viruses are difficult to eradicate due to the establishment of latency after exposure (Nandi *et al.*, 2009). Times of stress, such as those experienced during shipping and processing, can stimulate the virus to emerge from latency, inducing diseaseand further spreading the virus (Perez *et al.*, 2005). Bovine herpesvirus-1 is very common; cattle herds of over 400 head may have BHV-1 prevalence of over 85% (Raaperi *et al.*, 2010). Vaccines are readily available for

Corresponding Author: Sharon, K.P., Department of Animal and Range Sciences, Montana State University, Bozeman 59717, Montana, USA

BHV-1 that attempt to provide enough immunity to avoid reactivation of the disease. Although vaccination is common, humoral immunity is not always sufficient and reactivated-induced disease puts cattle at risk for developing BRD (Jones and Chowdhury, 2007).

There is evidence that lysine supplementation decreases the incidence and severity of herpesvirus-associated disease (Maggs *et al.*, 2000). *In vitro* studies have shown lysine has a replication-inhibiting capability of the herpes virus (Maggs *et al.*, 2000). Since IBR is the result of a herpes virus infection, there may be an altered response to the common IBR vaccination when lysine is supplemented at concentrations exceeding requirements of 17g/d determined by Hill *et al.* (2007). The objective of this study was to investigate the effects of a lysine supplementation on a modified-live viral respiratory vaccine.

2. MATERIALS AND METHODS

Procedures were reviewed and approved by the Montana State University Agriculture Care and Use Committee, 2012-AA01.

2.1. Animals, Treatments and Diets

Sixty-four Holstein calves (7±2 d of age; BW = 37±4.2 kg) were used in a completely randomized design. Sixteen calves were purchased from a commercial Montana dairy and were transported (approximately 30 min; 30 km) to the Montana State University facility after receiving their morning feeding. Forty-eight calves were purchased from a commercial Idaho calf ranch and were transported (approximately 6 h and 660 km) after their morning feeding to the Montana State University facility. Upon arrival, calves were weighed and randomly assigned to treatments including: LYS, lysine supplemented, CAS, casein supplemented and CON, control (no supplementation). Calves received either an IN, intranasal, or IM, intramuscular modified-live viral respiratory vaccination. Calves in the control group received no vaccination. Calves were housed in individual pens (1×2 m) fitted with feed and water buckets and nose guards to eliminate calf-to-calf contact. Pens were concrete-surfaced covered with rubber mats. Crates were maintained inside at the Bozeman Agriculture and Teaching (BART) Farm with a mean temperature of 15°C.

All calves were fed a commercial milk replacer (Vigortone, Nurture Pinnacle, Brookville, OH; DM: 95.7%; CP: 26.6%; crude fat: 18.7%; ash: 6.4%) at 0.45

kg/d per calf twice daily at 0700 and 1900 for 42 d. Milk replacer was mixed based on weight and at a concentration of 12% powder (as-fed) (Milk Master, Poly Tank and Polydome, Litchfield, MN). Milk replacer was fed using 2L nursing bottles (Calf Nurser Bottle, Merrick, Inc., Vadnais Heights, NM) with a rubber teat (Milk Bar Bottle Nipple, Coburn Company, Whitewater, WI) and individual bottle holders in each pen. LYS calves received 8.5 g of L-lysine monohydrochloride (Global Bio-Chem, Hong Kong, China) twice daily allocated to individual bottles and mixed with milk replacer. LYS calves received a total of 17 g·head^{-1}·d^{-1} of lysine. CAS calves received 8.5 g of casein allocated to individual bottles and mixed with milk replacer twice daily. CAS calves received a total of 17 g·head^{-1}·d^{-1} of casein. CON calves received no supplementation. After each feeding, bottles and teats were washed with a soap, bleach and water mixture.

Additionally, calves were offered a commercial starter feed (Dairy Starter Pellet; Payback Feed, Sioux Falls, SD; DM, 97.8; ADF, 8.9; NDF, 21.9 and CP, 19.9%) beginning on d 3 with an initial offering of 0.2 kg·head^{-1}·d^{-1}. Amounts were increased to ensure free choice consumption. Dailyallotments of grain were fed starting at 0730 in buckets. Daily refusals were weighed prior to feeding. Offered and refused feed was weighed back daily. Each calf was supplied with a water bucket. Water was changed twice daily. Water buckets were washed with a soap, bleach and water mixture weekly.

2.2. Data Collection

Rectal temperatures were constantly recorded by means of an indwelling rectal temperature probes starting on d 28 and ending on d 42. Temperature data loggers were attached to the rectal probes. Temperatures were recorded every 5 min and averaged every hour for the duration of the study for statistical analyses.

Calves were vaccinated on d 35 at approximately 1000 via intranasalinoculation with 2 mL of a modified-live respiratory vaccine containing BHV-1 and PI3 (Nasalgen, Merck Animal Health, Summit, NJ) or intramuscularly with 2 mL of a pentavalent, modified-live respiratory vaccine containing BHV-1, BVDV1a, BVDV2a, PI3 and BRSV (Express 5, Boehringer Ingelheim Vetmedica, Inc., Saint Joseph, MO). Control Calves (CON) received no vaccination.

Body weights were obtained on d 0, 1, 7, 14, 21, 28, 35 and 42 prior to the 1900 feeding. Grab samples of the

grain and milk replacer diet were collected weekly for analysis. Ingredient samples were analyzed for NDF and ADF, CF, fat and CP (Dairy One Laboratory, Ithaca, NY).

Incidence of any illnesses was recorded daily for each animal. Calves that showed signs of morbidity were treated immediately. Treatment regimen consisted of administration of tulathromyocin (Draxxin; Pfizer Animal Health) at a rate of 1.1 mL^{-1} 45.3 kg (2.5 mg kg^{-1}) and flunixinmeglumine (Banamine; Merck Animal Health) at a rate of 1.0 mL^{-1} 45.3 kg (2.2 mg kg^{-1}). All treatments including d of treatment, type of treatment and amount of dosage were recorded. Documentation of death was recorded byanimal ID and date of death.

Blood samples were collected via jugular venipuncture on d 0, 7, 14, 21, 28, 35, 36, 37, 39 and 42 in vacuum tubes (Kendall Monojet; Covidien, Mansfield, Massachusetts) for all calves. Blood collection was conducted prior to the 1900 h feeding. Serum was centrifuged at 2,100×g for 15 min at 20°C and then stored frozen at -20°C until analyzed. Frozen serum was transported to the Montana State University Veterinary Diagnostic Lab (Bozeman) for determination of serum neutralizing antibodies for BHV-1, PI3, BVDV type I, BVDV type II and BRSV. Serum samples were analyzed for SUN content by a commercial assay kit (Urea Nitrogen Direct; Cat. # 0580-250; Stanbio Laboratory, Boerne, TX). Additionally, serum samples were analyzed for serum lysine and arginine concentrations by means of mass spectrometry.

2.3. Calculations and Statistical Analysis

All data was analyzed using the Proc Mixed procedures of SAS (SAS Institute Inc., Cary, NC). Each calf was treated as the experimental unit. Performance data were analyzed with a model that included treatment, block and calf. Temperature, antibody titer data, serum lysine and arginine and SUN data were analyzed with a model that included treatment, calf (treatment), day and treatment x day. Significance was indicated when p<0.05.

3. RESULTS

No differences (p>0.10) were observed for initial BW, final BW, ADG, DMI or overall G: F across treatments (LYS, CAS and CON) shown in **Table 1**.

No differences were observed among treatments (LYS, CAS and CON) in vaccinated calves for IN **Fig. 1** or IM in **Fig. 2** for rectal temperature (p>0.10) or IBR antibody titer levels (p>0.10), **Fig. 3**.

Serum urea nitrogen in **Fig. 4** and the ratio of lysine: Arginine, **Fig. 5 and 6**, was greater for LYS compared to CAS calves.

Table 1. Effect of supplemental lysine on performance of neonatal Holstein calves

Item	Treatment[1]			
	CAS	LYS	CON	SEM[2]
Calves	28	28	8	-
BW, kg[3]				
Initial	36.5	36.7	34.4	1.1
Final	56.9	59.3	52.4	2.6
Performance, d 0 to 42[3]				
ADG, kg	0.45	0.46	0.43	0.07
DMI, kg	0.66	0.70	0.60	0.13
G: F	0.94	0.65	0.69	0.25

[1]Treatments were supplementation: CAS = 17 g/d casein; LYS = 17g/d L-lysine monohydrochloride; CON = no supplementation
[2]Pooled SEM
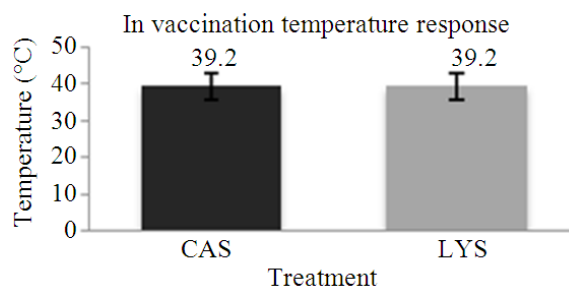[3]No difference (p>0.10)

Fig. 1. Effects of IN modified-live IBR-PI3 viral vaccination on rectal temperature in calves receiving 17 g/d of supplemental Lysine (LYS) or 17 g/d of Supplemental Casein (CAS) in neonatal Holstein calves. Averaged rectal temperature was not significant among treatments (p>0.10)
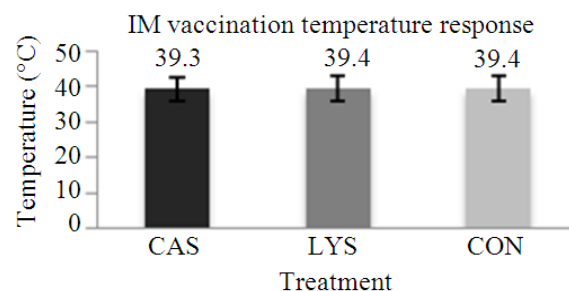

Fig. 2. Effects of IM modified-live IBR-PI3 viral vaccination on rectal temperature in calves receiving 17 g/d of supplemental Lysine (LYS), 17 g/d of supplemental Casein (CAS) or no supplementation (CON) in Holstein calves. Averaged rectal temperature was not significant among treatments (p>0.10)
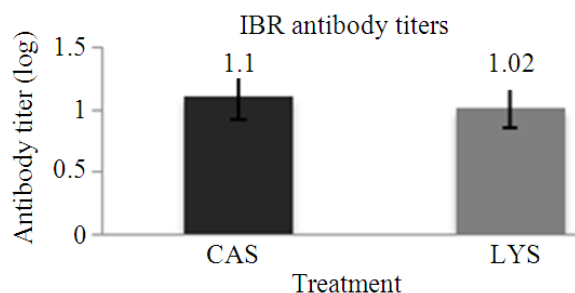
Fig. 3. Overall effects of Supplemental Lysine (LYS) or Casein (CAS) on IBR antibody titers following a modified-live viral respiratory vaccination in neonatal Holstein calves. No difference (p>0.10) between CAS and LYS calves was detected
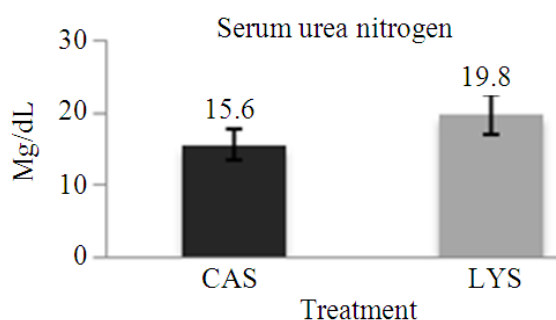

Fig. 4. Effects of supplemental Lysine (LYS) or supplemental Casein (CAS) on serum urea nitrogen in neonatal Holstein calves. Overall SUN was greater (p>0.05) in LYS compared to CAS calves
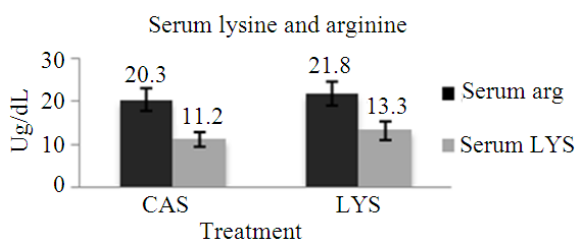

Fig. 5. Overall effects of Supplemental Lysine (LYS) or Supplemental Casein (CAS) on serum lysine and arginine levels in neonatal Holstein calves. No difference (p>0.10) between CAS and LYS calves was detected for serum lysine or serum arginine

4. DISCUSSION

Calf performance was not affected by treatment, suggesting that no treatment group had a nutritional advantage or disadvantage in mounting immunity in response to the vaccine.
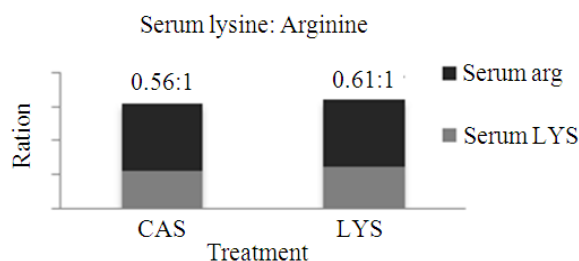

Fig. 6. Effects of supplemental Lysine (LYS) and supplemental Casein (CAS) on serum lysine: Arginine ratio in neonatal Holstein calves. Serum lysine: Arginine ratio was greater (p<0.05) in LYS compared to CAS calves

Available N was equal in CAS and LYS diets; casein was fed to ensure CAS and LYS diets were isonitrogenous. All calves received at least the recommended 17 g/d of lysine. Calves in the LYS group ingested double the amount of daily lysine requirements. Serum urea nitrogen levels were used to retrospetively diagnose biological responses to the added protein. Despite the diets of CAS and LYS groups being isonitrogenous, LYS treatment resulted in greater SUN levels. With all diets being formulated to meet nutritional requirements it can be proposed this additional lysine was simply biologically unessecary, resulting in increased SUN levels.

Lysine: Arginine ratios were greater in LYS calves group compared to CAS calves. This is important in the lysine replication inhibition of the herpesvirus. Lysine and arginine are very similar in molecular structure. For this reason, lysine may be substituted for arginine in biological pathways. This is important as the herpes virus requires arginine for replication (Maggs *et al*., 2000). Also contributing to lysine: Arginine ratios is lysine-arginine antagonism. Lysine-arginine antagonism has been studied in many species, although the complete mechanism has not been fully elucidated. Proposed mechanisms include an increase in renal arginase activity and inhibiton of reabsorption in the presence of high lysine levels (Chamruspollert *et al*., 2002). Abe *et al*. (2001) reported an imbalance of supplemental lysine would possibly occur only at levels of 48 to 64 g/d. Lysine-arginine antagonism was highly unlikey to have taken place in this study due to the acceptable levels of lysine fed.

Looking closer at immune reponse, no increase in rectal temperature was observed in vaccinated calves. Further, rectal temperature did not differ among vaccinated and non-vaccinated calves. Systemic fever both directly combats pathogens as well as stimulates

cellular immunity (Mace *et al*., 2011). The resulting immune stimulation results in accumulation and continued protection against a pathogen. An absence of febrile response suggests a lack of immune response to the vaccine. This was supported by observing no difference in antibody titers among vaccinated and non-vaccinated calves.

Factors influencing immune response in young calves have been well studied. Vaccine efficacy is unreliable in the presence of maternal antibodies. Seropositivity resulting from maternal antibodies may inhibit a response to vaccination early in life (Ellis *et al*., 2001; Endsley *et al*., 2003; Fulton *et al*., 2004). Although vaccinations were administered four weeks after arrival, maternal antibodies may have been a factor, reducing the immune response, febrile and antibody titer response in vaccinated animals. IBR antibody titers showed no difference among supplemented and control calves, or vaccinated and non-vaccinated calves. It is possible a difference in antibody titers could have been detected by sampling at a later date after vaccination. Kahrs (2001) along with Ezzi *et al*. (2011) reported calves can lose maternal antibodies as early as one month of age, but can have titers to BHV-1 up to six months of age.

These results ageed with Ellis *et al*. (2010) reporting reduced efficacy of an IN vaccination to a bovine respiratory syncytial virus challenge in seropositive animals compared to seronegative animals. Further, Patel and Didlick (2004) reported no increase in antibody titers in calves receiving a modified-live BRSV vaccine.

This study agrees with previous research in that maternal antibodies influence vaccination efficacy and should be kept in mind when developing vaccination protocol.

5. CONCLUSION

Bovine herpesvirus-1 is a significant pathogen contributing to economic loss in the cattle industy. Although no alterations in febrile response or IBR antibody titer levels, supplememtal lysine will alter serum lysine: Arginine concentrations in neonatal calves. These results suggest that it is possible to manipulate *in vivo* serum lysine concentrations, which could affect a replicating herpes virus. *In vitro* work has shown the replication inhibition of a herpesvirus in the presence of increased lysine levels (Maggs *et al*., 2000).

To further investigate the effects of lysine supplementation and herpes virus replication a live viral challenge may be valuable. Additionally, mature cattle may prove to be a better model in a vaccination challenge, removing the risk of maternal antibody interference. The results from this study encourage further work in exploration into bovine herpesvirus and lysine supplementation.

6. ACKNOWLEDGMENT

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