Oregon State University

Beef Cattle Sciences

# Beef Research Report

## 2012 Edition

Oregon State University | Extension Service

Oregon State University

BEEF083

Beef Cattle Sciences

Beef Research Report

# Effects of α-Linolenic and Linoleic Acids on Reproductive Performance of Replacement Beef Heifers[1]

E. Thompson [2], C. Muller [2], and R. F. Cooke [3]

## Synopsis

Our data demonstrates that feeding polyunsaturated fatty acids around the time of artificial insemination has the potential to increase reproductive performance by increasing circulating progesterone.

## Summary

The objective of this study was to determine if supplementing linoleic or α-linolenic acids prior to and after timed artificial insemination (TAI) alters conception rates, supplement intake, and serum progesterone (P4) concentrations in pre-parturient beef heifers. Fifty-four Angus-cross heifers (age 381 ± 10.2 days) were randomly assigned to one of the following dietary supplement groups: 1) barley and soybean meal (CON); 2) CON with 2% of DMI as flaxseed oil (α-linolenic acid; F2); 3) CON with 4% of DMI as flaxseed oil (F4); and 4) CON with 4% of DMI as safflower oil (linoleic acid; S4). Heifers were fed individually once per day with orts quantified. Supplement intake (DMISUPP) was equal to 25% of estimated total intake, based on 2.5% of BW. Heifers were synchronized using the 14-day CIDR®-PG method. Data was analyzed as a completely randomized design with heifer as the experimental unit and means were compared using the following contrasts: Oil vs. No-Oil, F2 vs. F4, and F4 vs. S4. The TAI conception rates were 57%, 62%, 64%, and 62% for CON, F2, F4, and S4, respectively. No differences in TAI conception or overall conception ($P > 0.10$) were observed for any comparison. On day 7, 15, and 22, heifers fed oil had higher P4 than those on CON ($P = 0.01$, 0.01, and 0.03; respectively), but no differences ($P > 0.10$) among oil treatments. From day -17 to d 21, F4 heifers had lower DMISUPP which translated into an overall decrease of DMISUPP among all contrasts ($P < 0.05$). Feeding α-linolenic or linoleic acid to heifers prior to and after TAI increases P4, but does not affect TAI or overall conception rates.

## Introduction

The primary goal of any beef cow-calf operation is to produce a calf from each cow every 12 months or less, starting at two years of age. Open, or un-bred, cows account for the majority of herd losses each year at 17% (Massey, 1993). Cattle producers can look for better ways to increase their calf crop by understanding physiological processes that occur around the time of conception. It is known that feeding polyunsaturated fatty acids can increase reproduction rates in cattle by increasing progesterone concentration in the blood (Santos, et al., 2008). Progesterone from the cow's corpus luteum (CL) is necessary for maintenance of pregnancy before fetal tissues take over hormonal control. Alpha-linolenic acid, present in flaxseed oil, is able to reduce prostaglandin synthesis from ovarian and uterine endometrial cells (Mattos, et al.,

1. This document is part of the Oregon State University – 2012 Beef Research Report. Please visit the Beef Cattle Sciences website at http://beefcattle.ans.oregonstate.edu.
2. Oregon State University, Eastern Oregon Agricultural Research Center-Union Station, Union, OR 97883.
3. Oregon State University - EOARC, Burns 97720. Email: reinaldo.cooke@oregonstate.edu.

2000), preventing the CL from regressing and the potential loss of pregnancy. Conversely, when metabolized by the body, linoleic acid provides a prostaglandin's precursor, arachdonic acid (Hess, et al., 2007).

The effect of α-linolenic acid has not been extensively studied in beef cattle but similar studies in dairy cattle provide insight to the benefits of α-linolenic acid in the diet on reproductive performance. Cows supplemented with α-linolenic acid are 1.5 times more likely to have conceived by day 41 post AI compared to those supplemented with palm oil (Santos, et al., 2008). Therefore, we hypothesize that feeding α-linolenic acid around TAI will increase the reproductive performance by increasing serum progesterone concentration (P4).

The objective of this study was to determine the impact of supplemental oil fed, including type of oil, prior to and after TAI on conception rates, P4, and supplemental intake.

## Materials and Methods

Use of live animals was approved through Oregon State University's IACUC. Fifty four Angus-cross heifers (mean weight 777 ± 56 lbs), were stratified by age (mean age 381 ± 10.2day) and randomly assigned to one of the following supplement groups: 1) barley and soybean meal (CON, n = 14); 2) CON with 2% of estimated DMI as flaxseed oil (α-linolenic acid; F2, n = 13); 3) CON with 4% of estimated DMI as flaxseed oil (F4, n = 14); and 4) CON with 4% of estimated DMI as safflower oil (linoleic acid; S4, n = 13). Supplements were formulated to be iso-nitrogenous (18.25% CP) (DM basis; Table 1). Grass hay, loose mineral, and water were provided ad libitum throughout the trial. Heifers received 25% of their daily DMI as supplement from day -25 to day 21, with day -25 through day -22 designated as the adaptation period. Daily intake was estimated as 2.5% of their individual BW. Prior to initiation of supplementation, all heifers were bled once every seven days for three consecutive weeks to determine estrus cyclicity. Body weights were collected during blood collection and diets adjusted appropriately on day -1. Health was monitored daily and treatment administered if necessary. Pregnancy status was determined by transrectal ultrasonography at 60 days post-TAI. Blood samples were analyzed for serum progesterone concentrations using an ELISA assay on day -50, -42, -36, 7, 15, 21, and 29. (Galvão, et al., 2004). From day -22 through day 21, orts were measured and nutritional analysis performed.

### *Statistical analysis*

Data were analyzed as a completely randomized design with heifer as the experimental unit, and means were compared using the following contrasts: Oil (means of F2, F4, and S4) vs. No-Oil (CON), F2 vs. F4, and F4 vs. S4.

**Table 1.** Supplement Analysis (% composition of supplement on a DM Basis).

| | Dietary Supplement | | | |
|---|---|---|---|---|
| **Item** | **CON** | **F2** | **F4** | **S4** |
| **Barley** | 83.7 | 73.42 | 63.49 | 63.49 |
| **SBM** | 16.3 | 18.69 | 20.7 | 20.7 |
| **Flaxseed Oil** | 0 | 7.89 | 15.81 | 0 |
| **Safflower Oil** | 0 | 0 | 0 | 15.81 |
| **DM**[1] | 90.63 | 91.2 | 91.8 | 91.8 |
| **CP**[1] | 18.13 | 18.2 | 18.11 | 18.11 |
| **NDF**[2] | 18.21 | 16.37 | 14.56 | 14.56 |
| **ADF**[2] | 6.84 | 6.26 | 5.69 | 5.69 |
| **NEm (Mcal/cwt)**[2] | 96.28 | 105.79 | 115.32 | 115.32 |
| **NE (Mcal/cwt)**[2] | 64.53 | 72.15 | 79.78 | 79.78 |

[1] % Dry matter (DM) and % crude protein (CP) are determined via laboratory.
[2] % Neutral detergent fiber (NDF), % acid detergent fiber (ADF), net energy maintenance (NEm), and net energy gain (NEg) are from NRC 1996.

## Results

No differences in TAI conception or overall conception rates occurred within any contrasts ($P$ = 0.984, 0.8464, and 0.8844). Figures 1 and 2 show that on day 7, 15, and 22, heifers supplemented with oil had higher P4 than those on CON ($P$ = 0.01, 0.01, and 0.03; respectively), but no differences ($P$ > 0.10) were detected for F2 vs. F4 and F4 vs. S4. On day 28, there were no differences ($P$ > 0.05) in P4 among contrasts. There were no differences ($P$ > 0.10) in DMISUPP among contrasts during the first days of acclimation to supplements. From day -17 to day 21, F4 heifers had lower DMISUPP which translated into an overall decrease of DMISUPP among all contrasts ($P$ < 0.05). Feeding α-linolenic

or linoleic acid to heifers prior to and after TAI increases P4, but does not affect TAI or overall conception rates.

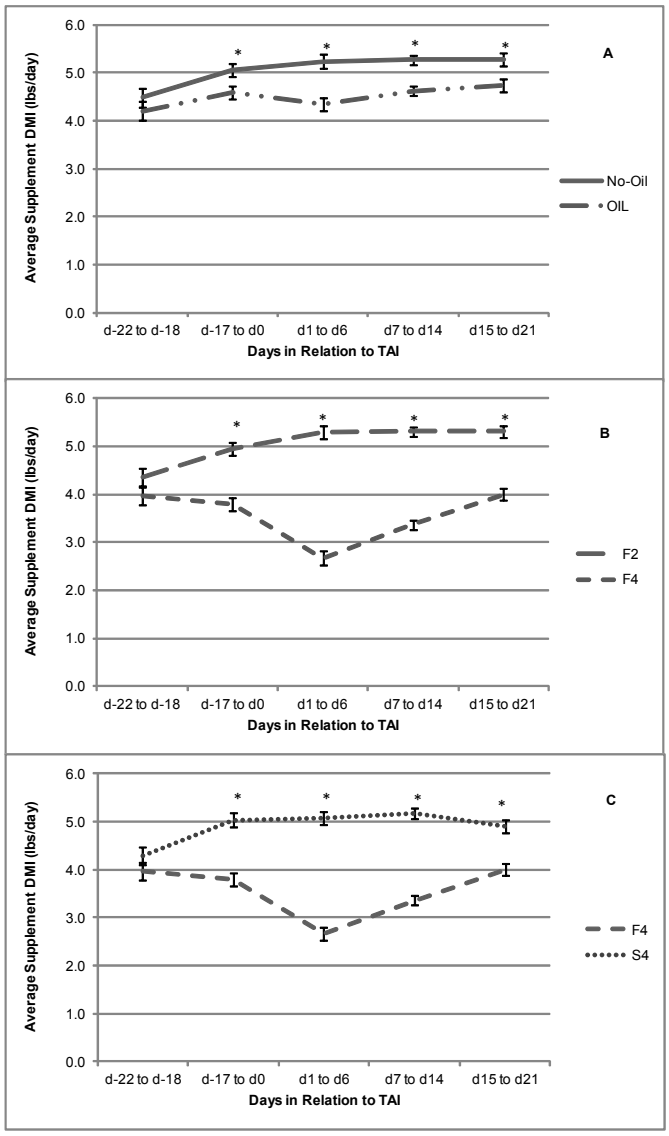


**Figure 1.** Supplemental DMI (lbs). Panel A: Oil vs. No-Oil. Panel B: F2 vs. F4. Panel C: F4 vs. S4. *$P < 0.01$ (Within time period).

## Conclusions

The results obtained from this study gave insight to the possibility of a negative feedback associated with the supplemental ingestion of high levels of α-linolenic acid in heifers. This depression in intake, which was only seen in the high α-linolenic acid group, became significant starting 8 days (including acclimation time of 3 days) after initiation of treatment and was only seen in the F4 group, indicating the source, rather than the quantity, could play a role in intake. Moreover, despite the oil source, heifers supplemented with all types and

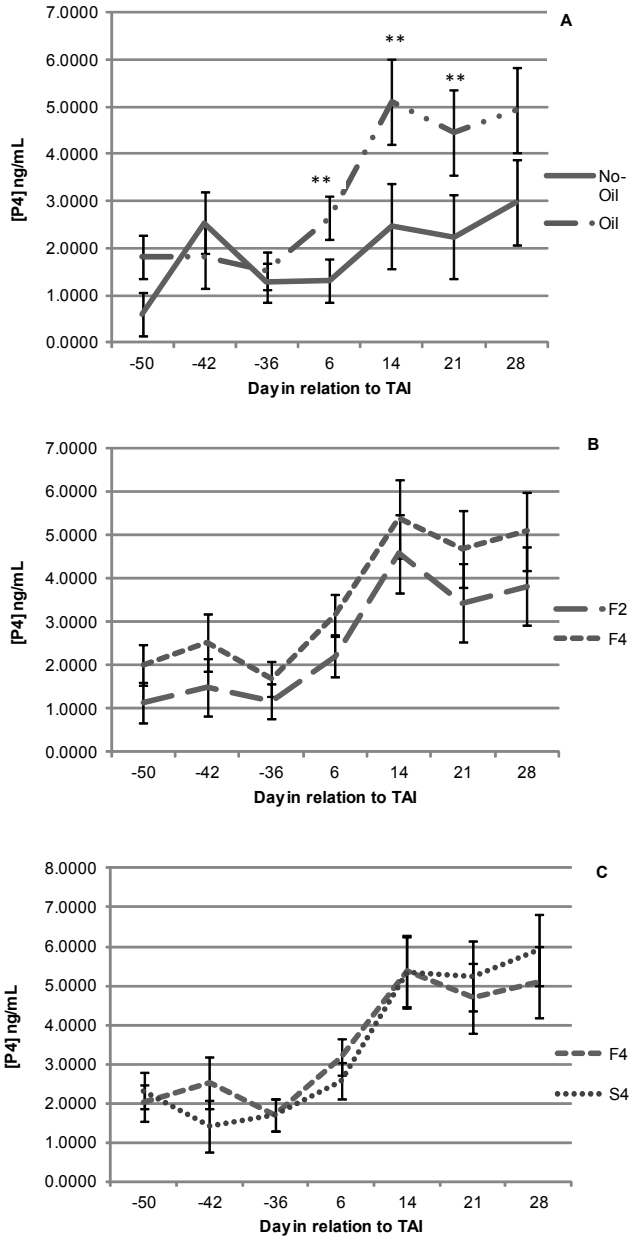


**Figure 2**. Serum Progesterone Concentrations (ng/mL). Panel A: Oil vs. No-Oil. Panel B: F2 vs. F4. Panel C: F4 vs. S4. **$P < 0.05$ (Within time period).

quantities of oil had higher serum P4 than those on the control diet. Feeding high oil diets, including linoleic acid, increases serum lipoproteins and cholesterol (Garcia et al. 2003), and this could be one cause for the increase in P4 in the S4 group. The increase in circulating cholesterol could be overriding PGF2α synthesis from endometrial cells because cholesterol is the primary precursor for progesterone synthesis and progesterone and

prostaglandin are inversely related (Santos, et al, 2008, Mattos et al., 2000).

Further research should be conducted to determine if a metabolic or a hormonal feedback is associated with the depression in consumption seen for the high α-linolenic acid treatment.

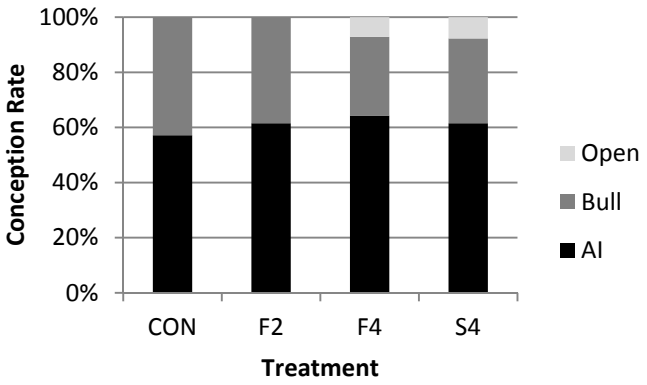


**Figure 3.** Conception status by treatment group. No significant differences within contrasts (*P*> 0.10).

## Acknowledgements

We would like to thank Ross Bowmar and ADM for the donation of three, 50 gallon drums of flaxseed oil. In addition, we would like to thank Chad Mueller, Reinaldo Cooke, Tim DelCurto, Grace Deboot, Kenny Fite, and Mark Fite for their assistance in conducting the field work and their help in the lab. Without them, this research could not have taken place.

## Literature Cited

Galvão et al. 2004. J. Anim. Sci. 82:3508–3517.

Garcia et al. 2003. J. Anim. Sci. 81:261-268.

Hess et al. 2007 J. Anim. Sci. 86: 188-204.

Massey et al. 1993. University of Missouri Extension. Dept of Anim. Sci.

Mattos et al. 2000. Rev. Repro. 5:38-45.

Santos et al. 2008. Repro. Dom. Anim. 43:23-30.

Oregon State University

BEEF084

Beef Cattle Sciences

Beef Research Report

# Effects of Acclimation to Human Handling on Temperament, Physiological Responses, and Performance of Beef Steers during Feedlot Receiving[1]

C. Francisco[2,3], R. F. Cooke[2], R. Marques[2], F. T. Cooke[2], T. A. Guarnieri Filho[3], and D. W. Bohnert[2]

## Synopsis

Acclimation of feeder steers to human handling after weaning improves cattle temperament but impairs performance during feedlot receiving.

## Summary

The objective was to compare temperament, plasma concentrations of cortisol and acute-phase proteins, and performance during feedlot receiving of Angus × Hereford steers acclimated or not to human handling. Sixty steers were initially evaluated, within 30 d after weaning, for BW and temperament score (average chute score and exit velocity score; d -30). On d -28, steers were ranked BW and temperament score, and randomly assigned to receive or not (control) the acclimation treatment. During the acclimation phase (d -28 to 0), steers were maintained in 2 pastures according to treatment, and acclimated steers were exposed to a handling process twice weekly (Tuesdays and Thursdays). The acclimation treatment was applied individually to steers by processing them through a handling facility, whereas control steers remained undisturbed on pasture. On d 0, all steers were loaded into a commercial livestock trailer, transported for 24 h, and returned to the research facility (d 1). Upon arrival, steers were ranked by BW within treatment, and randomly assigned to 20 feedlot pens. Total DMI was evaluated daily from d 1 to d 28, and shrunk BW was collected on d -31, 1, and 29 for ADG calculation. Blood samples were collected on d -28, 0 (prior to loading), 1 (immediately upon arrival), 4, 7, 10, 14, 21, and 28 for determination of cortisol, ceruloplasmin, and haptoglobin. Steer temperament was assessed again on d 0. During the acclimation phase (d -28 to 0), no treatment effects were detected ($P = 0.14$) on steer ADG. Acclimated steers had reduced chute score compared with control on d 0 ($P = 0.01$). During feedlot receiving (d 1 to 28), acclimated steers had reduced ADG ($P < 0.01$), DMI ($P = 0.07$), and G:F ($P = 0.03$) compared with control. Acclimated steers had greater plasma cortisol on d 1 ($P = 0.06$), greater haptoglobin on d 4 ($P = 0.04$), and greater ceruloplasmin from d 0 to 10 ($P \leq 0.04$) compared with control. In conclusion, steers exposed to the acclimation process had greater stress-induced cortisol and acute-phase protein responses, resulting in decreased performance during feedlot receiving.

## Introduction

Temperament is defined as the behavioral responses of cattle when exposed to human handling (Burrow, 1997; Burrow e Corbert, 2000; Curley et al., 2006). Animals with aggressive temperament display nervous or agitated responses during human

1. This document is part of the Oregon State University – 2012 Beef Research Report. Please visit the Beef Cattle Sciences website at http://beefcattle.ans.oregonstate.edu.
2. Oregon State University - Eastern Oregon Agricultural Research Center, Burns, OR.
3. UNESP – Universidade Estadual Paulista, FMVZ/DPA, Botucatu, São Paulo, Brazil.

contact or any other handling procedures. Besides personnel security and animal welfare, temperament has significant implications on beef cattle performance. Our research group was the first to report that beef cows with aggressive temperament have impaired reproductive performance compared to cows with adequate temperament (Cooke et al., 2009a; Cooke et al., 2012). In addition, our group recently reported that aggressive beef calves are lighter and consequently less valuable if sold at weaning, and also have decreased growth rates during the feedlot, resulting in reduced carcass marbling, carcass weight, and final carcass value if marketed upon slaughter (Cooke et al., 011). Therefore, cattle temperament should be used as a management decision criterion to enhance overall productivity and safety of beef operations.

Temperament of feeder calves can be improved by two main strategies. The first is to select the cowherd for calm temperament, which should also benefit the calf crop given that temperament is a heritable trait (Fordyce et al., 1988). Second, recent studies from our group demonstrated that acclimation of young cattle to human handling improved their temperament and enhanced their productivity (Cooke et al., 2009b, Cooke et al., 2012). However, this method was only tested with replacement heifers by evaluating their reproductive development. Based on this information, we hypothesized that acclimation to human interaction after weaning will also improve temperament and feedlot productivity of feeder steers. Therefore, the objective of this study was to compare temperament, plasma concentrations of cortisol and acute-phase proteins, and performance during feedlot receiving of steers acclimated or not to human interaction after weaning.

## Materials and Methods

The study was conducted at the Eastern Oregon Agricultural Research Center, Burns. Animals utilized were cared for according to an approved Oregon State University Animal Care and Use protocol. Sixty Angus x Hereford steers were initially evaluated, within 30 d after weaning, for BW and temperament score (average chute score and exit velocity score). Chute score was assessed based on a 5- point scale according to the method described by Arthington et al. (2008). Exit velocity was assessed by determining the speed of the steer exiting the squeeze chute by measuring rate of travel over a 1.8-m distance with an infrared sensor (FarmTek Inc., North Wylie, TX). Further, steers were divided in quintiles and assigned an exit velocity score on a 5-point scale (1 = slowest quintile; 5 = cows within the fastest quintile). On d -28, steers were ranked BW and temperament score and randomly assigned to receive or not (control) the acclimation treatment. Steers were maintained on separate meadow foxtail (Alopecurus pratensis L.) pastures (30 steers/pasture) according to treatment, and received supplemental alfalfa hay 3 times weekly to sustain a growth rate of approximately 0.5 kg/d. The acclimation treatment was applied individually to steers by processing them through a handling facility, twice week (Tuesdays and Thursdays) for 4 weeks, while control steers remained undisturbed on pasture. In addition, during feeding procedures, the technician walked among steers assigned to the acclimation treatment for 15 min to further expose them to human interaction, whereas the same procedure was not applied to control steers.

On d 0, all steers were loaded into a commercial livestock trailer, transported for 24 h for a total of 1,200 km, and returned to the research facility on 1. Upon arrival, steers were ranked by BW within treatment, and randomly assigned to 20 feedlot pens (10 pens/treatment; 3 steers/pen). All pens received 2.5 kg/steer daily of a concentrate (86% corn; 14% soybean meal), whereas meadow foxtail hay was offered in amounts to ensure ad libitum access. Total DMI was evaluated daily from d 1 to d 28, and shrunk BW was collected on d -31, 1, and 29 for ADG calculation. Total DMI and BW gain from d 1 to 28 were used to calculate feedlot receiving G:F.

Blood samples were collected on d -28, 0 (prior to loading), 1 (immediately upon arrival), 4, 7, 10, 14, 21, and 28 via jugular venipuncture into commercial blood collection tubes containing sodium heparin (Vacutainer, 10 mL; Becton Dickinson, Franklin Lakes, NJ). Steer rectal temperature (RT) was measured by digital thermometer (GLA M750 digital thermometer; GLA Agricultural Electronics, San Luis Obispo, CA) concurrently with each blood collection. All blood samples were harvested for plasma and stored at −80°C until assayed for concentrations of cortisol (Endocrine Technologies Inc., Newark, CA), haptoglobin (Cooke and Arthington, 2012) and ceruloplasmin (Demetriou et al., 1974).

Data were analyzed using the PROC MIXED procedure of SAS (SAS Inst., Inc., Cary, NC) and Satterthwaite approximation to determine

the denominator df for the tests of fixed effects. The model statement used for ADG contained the effects of treatment. Data were analyzed using steer(treatment × pen) as random variable. The model statement used for DMI and G:F contained the effects of treatment, as well as day and the resultant interaction for DMI only. Data were analyzed using pen(treatment) as the random variable. The model statement used for temperament and physiological measurements contained the effects of treatment, day, and the resultant interactions. Data were analyzed using steer(treatment × pen) as the random variable. The specified term for repeated statements was day, pen(treatment) or steer(treatment × pen) as subject for DMI or temperament and physiological variables, respectively, and the covariance structure utilized was based on the Akaike information criterion. Results are reported as least square means and were separated using LSD. Significance was set at $P \leq 0.05$ and tendencies were determined if $P > 0.05$ and $P \leq 0.10$. Results are reported according to treatment effects if no interactions were significant, or according to the highest-order interaction detected.

## Results and Discussion

During the acclimation phase (d -28 to 0), no treatment effects were detected ($P = 0.14$) on steer ADG (Table 1). On d 0, acclimated steers had reduced ($P = 0.01$) chute score, and tended ($P = 0.08$) to have reduced temperament score compared to control cohorts (Table 1). However, during feedlot receiving (d 1 to d 28), acclimated steers tended ($P = 0.07$) to have reduced DMI, and had reduced ($P \leq 0.03$) ADG and G:F compared to control cohorts (Table 1). Similarly to our previous work (Cooke et al., 2009b, Cooke et al., 2012), acclimation to handling improved temperament of growing cattle. However, steers exposed to the acclimation process experienced reduced feedlot receiving performance compared to control cohorts. This performance outcome was unexpected given that a similar acclimation process enhanced reproductive and overall performance of replacement heifers (Cooke et al., 2009b, Cooke et al., 2012).

**Table 1.** Temperament and feedlot receiving performance of beef steers exposed (ACC) or not (CON) to handling acclimation procedures. [1]

| Item | ACC | CON | SEM | *P*= |
|---|---|---|---|---|
| Temperament variables[2] | | | | |
| Chute score | 1.63 | 2.07 | 0.12 | 0.01 |
| Exit velocity, m/s | 1.97 | 2.28 | 0.16 | 0.18 |
| Temperament score | 2.21 | 2.63 | 0.16 | 0.08 |
| Performance variables | | | | |
| Total DMI, kg/d | 7.09 | 7.40 | 0.11 | 0.07 |
| Acclimation ADG, [3] kg/d | 0.27 | 0.38 | 0.06 | 0.14 |
| Receiving ADG, [4] kg/d | 1.13 | 1.32 | 0.05 | < 0.01 |
| G:F, [5] kg/kg | 166 | 185 | 6 | 0.03 |

[1] Acclimated steers were exposed to a handling process twice week for 4 wk (d -28 to 0), which was applied individually to steers by processing them through a handling facility. Control steers remained undisturbed on pasture.
[2] Obtained on d 0. Chute score (1-5 scale), exit velocity, and temperament score were calculated according to the techniques described by Cooke et al. (2011).
[3] Calculated using shrunk values obtained on d -31 and d1.
[4] Calculated using shrunk values obtained on d 1 and d29.
[5] Calculating using total DMI and BW gain from d 1 to d29.

No treatment effects were detected ($P > 0.24$; data not shown) for RT (38.84 vs. 39.03ºC for ACC and CON steers, respectively; SEM = 0.07). Treatment x day interactions were detected for cortisol, haptoglobin, and ceruloplasmin ($P \leq 0.05$). Acclimated steers had greater plasma cortisol on d 1 ($P = 0.05$), greater haptoglobin on d 4 ($P = 0.04$), and greater ceruloplasmin from d 0 to 10 ($P \leq 0.04$) compared with control steers (Figure 1). Contrary to these outcomes, replacement heifers assigned to a similar acclimation process had reduced cortisol (Cooke et al., 2009b) and haptoglobin (Cooke et al., 2012). The exact reasons for the different outcomes to the acclimation process reported herein and by our previous work are unknown and deserve further investigation. Nevertheless, steers assigned to the acclimation process had a more severe neuroendocrine stress and acute-phase protein response upon transportation and feedlot entry compared to control cohorts, which likely contributed to their reduced DMI, G:F, and ADG during feedlot receiving (Arthington et al., 2003; Qiu et al., 2007; Araujo et al., 2010).

## Conclusions

Acclimation of feeder steers to human handling after weaning improved cattle temperament but increased the neuroendocrine stress and acute-phase responses following transport and feedlot entry, resulting in decreased performance during feedlot receiving.

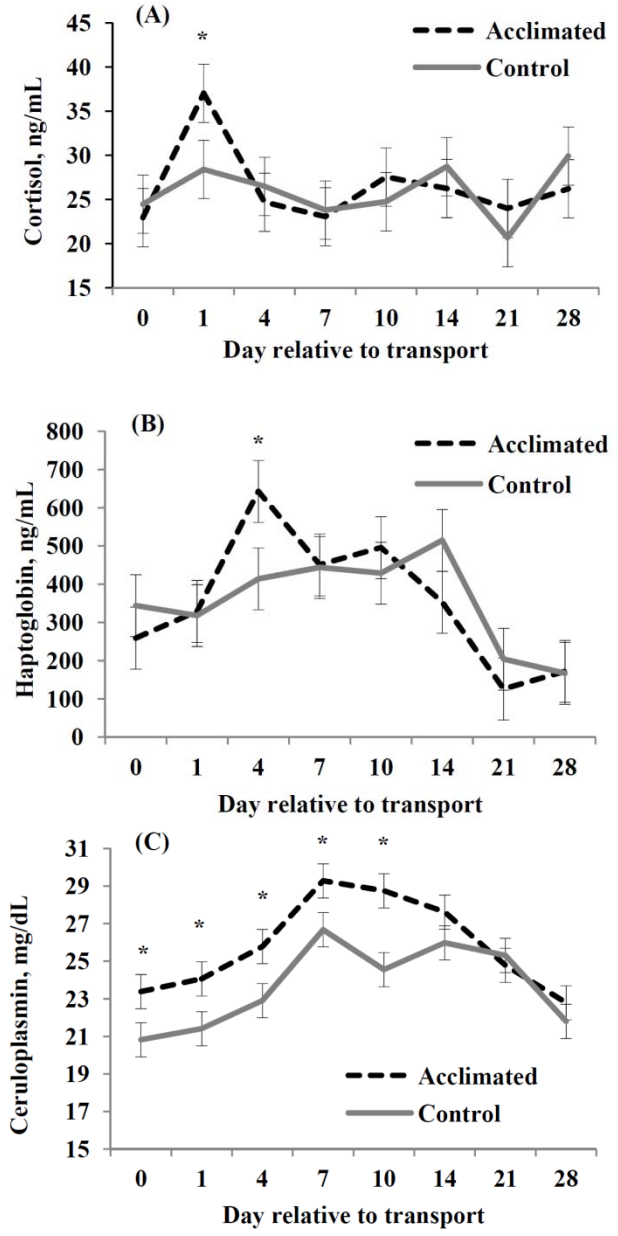


**Figure 1**. Plasma concentrations of cortisol (Panel A), haptoglobin (Panel B), and ceruloplasmin (Panel C) during feedlot receiving (d 1 to d 28) of beef steers exposed (acclimated) or not (control) to handling acclimation procedures (d -28 to 0) and transported for 24 h (d 0 to d 1). A treatment × day interaction was detected ($P \leq 0.05$) for all variables. Treatment comparison within day; * $P < 0.05$.

## Literature Cited

Araujo et al. 2010. J. Anim. Sci. 87:4125-4132.

Arthington et al. 2003. J. Anim. Sci. 81:1120-1125.

Arthington et al. 2008. J. Anim. Sci. 86:2016–2023.

Burrow. 2001. Anim. Breed. 65:478–495.

Burrow and Corbert, 2000. Aust. J. Agric. Res. 51:155-162.

Cooke et al. 2009a. J. Anim. Sci. 87:4125-4132.

Cooke et al. 2009b. J. Anim. Sci. 87:3403-3412.

Cooke et al. 2011. Livest. Sci. 142:108-113.

Cooke and Arthington. 2012. J. Anim. Physiol. Anim. Nutr. doi: 10.1111/j.1439-0396.2012.01298.

Cooke et al. 2012. J. Anim. Sci. doi:10.2527/jas.2012-4768.

Curley Jr et al. 2006. J. Anim. Sci. 84: 3100-3013.

Demetriou et al. 1974. Clinical Chemistry: Principles and Techniques.

Fordyce et al. 1988. Aust. J. Exp. Agric. 28:683.

Qiu et al. 2007. J. Anim. Sci. 85:2367-2374.

Oregon State University

BEEF085

Beef Cattle Sciences

Beef Research Report

# Plasma Progesterone Concentration in Beef Heifers Receiving Exogenous Glucose, Insulin, or Bovine Somatotropin[1]

B. I. Cappellozza[2], R. F. Cooke[2], F. N. T. Cooke[2], T. A. Guarnieri Filho[3], D. W. Bohnert[2], and J. L. M. Vasconcelos[3]

## Synopsis

The effects of insulin on hepatic progesterone degradation and circulating progesterone concentrations in bovine females in adequate nutritional status are dependent on circulating glucose, but not IGF-I.

## Summary

Three experiments were conducted to evaluate plasma concentrations of glucose, insulin, IGF-I, and progesterone (P4) in pubertal heifers receiving exogenous glucose, insulin, or sometribove zinc. In Exp. 1, 8 pubertal nulliparous Angus x Hereford heifers were randomly assigned to receive, in a crossover design containing 2 periods of 10 h each, infusions of: insulin (1 µg/kg of BW; INS) or saline (0.9%; SAL). Treatments were administered in 7 applications 45 min apart. Heifers receiving INS had greater ($P < 0.01$) plasma insulin, reduced ($P \leq 0.04$) plasma glucose and IGF-I, but similar ($P = 0.62$) plasma P4 concentrations compared with SAL heifers. In Exp. 2, the same heifers were assigned to receive, in a similar experimental design as Exp. 1, infusions of: insulin (1 µg/kg of BW) and glucose (0.5 g/kg of BW; INS+G) or SAL. Heifers receiving INS+G had greater ($P \leq 0.02$) plasma insulin, glucose, and P4, but reduced ($P = 0.01$) plasma IGF-I concentrations compared with SAL heifers. In Exp.3, the same heifers were assigned to receive, in a crossover design containing 2 periods of 14 d, injections of: 250 mg sometribove zinc (BST) or SAL. Heifers receiving BST had greater ($P < 0.01$) plasma glucose, IGF-I, and similar ($P \geq 0.67$) plasma insulin and P4. Results from this series of experiments suggest that concurrent increases in glucose and insulin are required to reduce hepatic catabolism and increase plasma concentrations of P4 in bovine females.

## Introduction

Nutrition, more specifically energy intake, is the environmental factor that most influences the reproductive function in beef females (Mass, 1987), including hastened attainment of puberty, decreased postpartum interval, and greater pregnancy rates (Wiltbank et al., 1962; Schillo et al., 1992; Pescara et al., 2010). Moreover, beneficial effects of energy intake on cattle reproduction are regulated, at least partially, by circulating hormones and metabolites such as glucose, insulin, and IGF-I (Wettemann et al., 2003). Our research group demonstrated that insulin modulates circulating concentrations of progesterone (P4; Lopes et al., 2009) by stimulating luteal P4 synthesis (Spicer and Echternkamp, 1995) and/or alleviating hepatic steroid catabolism (Lemley et al., 2008). In another study, Vieira et al.

1. This document is part of the Oregon State University – 2012 Beef Research Report. Please visit the Beef Cattle Sciences website at http://beefcattle.ans.oregonstate.edu.
2. Oregon State University - EOARC, Burns 97720.
3. UNESP – Universidade Estadual Paulista, FMVZ/DPA, Botucatu, São Paulo, Brazil.

(2010) reported that cows in adequate nutritional status receiving intravenous (i.v.) glucose infusion to increase plasma insulin concentrations had greater plasma P4 concentrations compared with cohorts receiving saline, which was attributed to reduced hepatic P4 degradation given that cows were ovariectomized and supplemented with exogenous P4. Therefore, we hypothesized that the insulin-stimulated decrease in hepatic P4 catabolism may also be dependent on circulating glucose and IGF-I. Based on this rationale, 3 experiments were conducted to evaluate plasma concentrations of glucose, insulin, IGF-I, and P4 in beef females receiving exogenous insulin, insulin + glucose, or ST.

## Materials and Methods

*Experiment 1.* Eight pubertal, nulliparous Angus x Hereford heifers (initial BW = 452 ± 12 kg; initial age = 656 ± 7 d) were assigned to an estrus synchronization protocol (d -16 to 0 of the study). On d -16 heifers received a 100-µg treatment of GnRH (Cystorelin, Merial Ltd., Duluth, GA) and a controlled internal drug releasing device containing 1.38 g of P4 (CIDR, Pfizer Animal Health, New York, NY), PGF2α treatment (25 mg Lutalyse, Pfizer Animal Health) and CIDR removal on d -9, and a second GnRH treatment (100 µg) on d -7. On d 0, heifers received another PGF2α treatment (25 mg) and a CIDR that remained in heifers throughout Exp. 1 (d 0 to 14). Transrectal ultrasonography examinations were performed immediately and 48 h after the second GnRH (d -7) and PGF2α (d 0) treatments to verify ovulation and corpus luteum (CL) regression, respectively. All heifers utilized in this experiment responded to the hormonal treatment.

Heifer BW was recorded at the beginning and end of the experiment (d 0 and 14). On d 5, heifers were randomly assigned to receive, in a crossover design containing 2 periods of 10 h each (d 6 and 8): 1) i.v. insulin infusion (1 µg/kg of BW; INS), or 2) i.v. saline infusion (0.9%; SAL). Bovine insulin solution was dissolved into 10 mL of physiological saline immediately prior to infusions and administered via jugular venipuncture in 7 applications (0.15 µg/kg of BW per application) 45 min apart (0, 45, 90, 135, 180, 225, and 270 min), whereas SAL heifers concurrently received 10 mL of physiological saline. Blood samples were collected immediately before each infusion, as well as at -120, -60, 330, 390, and 450 min relative to first infusion. All heifers were fasted for 12 h prior to the beginning of each period.

*Experiment 2.* Immediately after the end of Exp.1 (d 14), the same heifers (mean BW = 456 ± 14 kg) received a new CIDR and evaluated via transrectal ultrasonography to confirm the absence of a CL. Heifer BW was recorded at the beginning and end of the experiment (d 14 and 28). On d 20, heifers were randomly assigned to receive, in a crossover design containing 2 periods of 10 h each (d 20 and 22): 1) i.v. infusion containing insulin (1 µg/kg of BW) and glucose (0.5 g/kg of BW; INS+G), or 2) i.v. saline infusion (0.9%; SAL). Glucose and bovine insulin solution were dissolved into 10 mL of physiological saline immediately prior to infusions. Similarly to Exp. 1, infusion was administered via jugular venipuncture in 7 applications (0.07 g/kg and 0.15 µg/kg of BW per application for glucose and insulin, respectively) 45 min apart. Blood samples were collected immediately before each infusion, as well as at -120, -60, 330, 390, and 450 min relative to the first infusion. As in Exp. 1, heifers were fasted for 12 h prior to the beginning and during the sampling.

*Experiment 3.* Immediately after the end of Exp. 2 (d 28), heifers (mean BW = 462 ± 14 kg) received a new CIDR and were evaluated via transrectal ultrasonography to confirm the absence of CL. Heifer BW was recorded at the beginning and end of the experiment (d 28 and 55). On d 28, heifers were randomly assigned to receive, in a crossover design containing 2 periods of 14 d each (d 28 to 42 and 42 to 56): 1) s.c. injection containing 250 mg sometribove zinc (BST; Posilac, Elanco, Greenfield, IN), or 2) s.c. saline injection (0.9%; SAL). Treatments were applied once, at 0800 h, during the first day of each period (d 28 and 42). Heifer also received a new CIDR at the beginning of the second period concurrently with treatment administration (d 42). Four blood samples were collected, 3 h apart (from 0900 to 1800 h) from heifers on d 33, 35, and 37 (period 1) and 47, 49, and 51 (period 2) of the experiment. Similarly to Exp. 1 and 2, all heifers were fasted for 12 h prior to the beginning and during each collection day.

*Diets*. During all experiments, all heifers were individually offered (as-fed basis) 12 kg of mixed alfalfa-grass hay, 1.0 kg of ground corn, and 0.5 kg of camelina meal in the morning (0700 h). Heifers also received a complete commercial mineral and vitamin mix and water for ad libitum consumption.

*Blood analysis.* All blood samples were collected via jugular venipuncture into commercial blood collection tubes (Vacutainer, 10 mL; Becton Dickinson, Franklin Lakes, NJ) containing sodium heparin, placed on ice immediately, and centrifuged at 2,400 × g for 30 min for plasma collection. Plasma was stored at −80°C until assayed for concentrations of glucose (#G7521; Pointe Scientific, Inc., Canton, MI), insulin (B1009; Endocrine Technologies Inc., Newark, CA), IGF-I (SG100; R&D Systems, Inc., Minneapolis, MN), and P4 (11-PROHU-E01; Alpco, Salem, NH).

*Statistical analysis.* All data were analyzed using the PROC MIXED procedure of SAS (SAS Inst., Inc., Cary, NC) and Satterthwaite approximation to determine the denominator degrees of freedom for the tests of fixed effects. Heifer was considered the experimental unit for all analysis. The model statement used for Exp. 1 and 2 contained the effects of treatment, time, the resultant interaction, in addition to period as independent variable. Heifer was used as random variable. The specified term for the repeated statement was time, and heifer (treatment × period) was included as subject. The model statement used for Exp. 3 contained effects of treatment, day, time, and all interactions, in addition to period as independent variable. Heifer was used as random variable. The specified term for the repeated statement was time, and heifer (treatment × day × period) was included as subject. All results are reported as least square means and separated using LSD. For all analysis, significance was set at $P \leq 0.05$, tendencies were determined if $P \leq 0.10$.

## Results

*Experiment 1.* Heifer BW did not change ($P = 0.51$; data not shown) during the experimental period, indicating that heifers were in adequate nutritional status. Mean plasma insulin concentration during the experimental period was greater ($P < 0.01$) for INS compared with SAL (Table 1). A treatment × time interaction was detected ($P = 0.01$) for plasma glucose (Figure 1). After the initial infusion, plasma glucose decreased for INS heifers (time effect; $P < 0.01$) and did not change for SAL heifers (time effect; $P = 0.53$). Moreover, mean plasma glucose concentration during the experimental period was reduced ($P < 0.01$; Table 1) for INS compared with SAL heifers. In agreement, Kegley et al. (2000) also reported that i.v. insulin infusion reduced circulating glucose concentrations in beef cattle, given that insulin directly estimates the uptake of glucose by body tissues (Nelson and Cox, 2005).

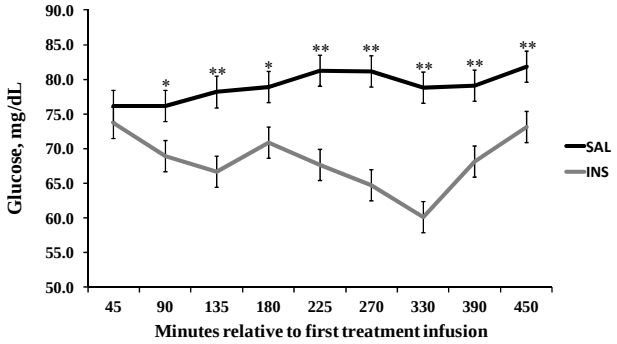


**Figure 1.** Plasma glucose concentrations of heifers receiving i.v. infusions containing saline (SAL) or insulin (INS). A treatment × time interaction was detected ($P < 0.01$). Treatments comparison within time: ** $P < 0.01$, * $P = 0.01$.

Mean plasma IGF-I concentration was reduced ($P = 0.04$) for INS heifers compared with SAL heifers during the experimental period (Table 1). The goal of Exp. 1 was o evaluate if insulin administration would increase plasma P4 concentrations in beef heifers in adequate nutrient balance, by reducing hepatic P4 catabolism, independently of circulating concentrations of glucose and IGF-I. However, no treatment effects were detected ($P = 0.62$) for plasma P4 concentrations (Table 1). Therefore, insulin itself may not be capable of alleviating hepatic P4 catabolism and consequently increasing circulating concentrations of this hormone. Accordingly, research studies documenting the role of insulin on hepatic expression of P4 catabolic enzymes (Lemley et al., 2008) and resultant plasma P4 concentrations (Vieira et al., 2010) included glucose infusion into the experimental design.

**Table 1.** Plasma concentrations of glucose, insulin, IGF-I, and P4 in beef heifers receiving i.v. infusion of insulin (INS) or saline (SAL) in Exp. 1.

| Item | INS | SAL | SEM | *P*-Value |
|---|---|---|---|---|
| Glucose, mg/dL | 68.20 | 79.00 | 1.30 | < 0.01 |
| Insulin, ng/mL | 1.40 | 0.99 | 0.10 | < 0.01 |
| IGF-I, ng/mL | 145.00 | 154.00 | 3.00 | 0.04 |
| P4, ng/mL | 3.74 | 3.84 | 0.27 | 0.65 |

*Experiment 2*. Similarly to Exp. 1, BW did not change ($P = 0.55$; data not shown) during the experimental period. As expected by the experimental design, mean plasma glucose and insulin concentrations during the experimental period were greater ($P \leq 0.01$) for INS+G compared with SAL heifers (Table 2) Similarly to Exp. 1, INS+G heifers had reduced ($P = 0.01$) mean plasma IGF-I concentrations compared with SAL heifers during the experimental period (Table 2).

**Table 2.** Plasma concentrations of glucose, insulin, IGF-I, and P4 in beef heifers receiving i.v. infusion containing insulin and glucose (INS+G) or saline (SAL) in Exp. 2.

| Item | INS | SAL | SEM | *P*-Value |
|---|---|---|---|---|
| Glucose, mg/dL | 133.90 | 76.80 | 16.40 | 0.01 |
| Insulin, ng/mL | 3.65 | 2.12 | 0.32 | < 0.01 |
| IGF-I, ng/mL | 134.00 | 142.00 | 2.00 | 0.01 |
| P4, ng/mL | 2.88 | 2.52 | 0.11 | 0.02 |

During the experimental period, INS+G heifers had greater ($P = 0.02$) mean P4 concentration compared with SAL heifers (Table 2). The goal of Exp. 2 was to evaluate if supplemental glucose modulates the effects of insulin infusion on plasma P4 concentrations by reducing hepatic P4 catabolism. In fact, we also expected that INS+G heifers would have greater plasma IGF-I, whereas IGF-I also influences hepatic function and could potentially modulate hepatic steroid catabolism (Jones and Clemmons, 1995). Nevertheless, results from Exp. 2 suggest that i.v. insulin infusion increased plasma P4 concentrations by reducing hepatic P4 catabolism only when supplemental glucose is provided. Therefore, results from Exp. 2 combined with those reported by Lemley et al. (2008) and Vieira et al. (2010) suggest that circulating glucose modulates the effects of insulin on hepatic steroid catabolism and subsequent circulating P4 concentrations in bovine females in adequate nutritional status.

*Experiment 3*. Similarly to Exp. 1 and 2, BW did not change ($P = 0.72$; data not shown) during the experimental period. As expected, BST heifers had greater ($P < 0.01$) mean plasma IGF-I concentrations compared with SAL heifers (Table 3), given that sometribove zinc has been shown to increase IGF-I synthesis and circulating concentrations in cattle (Bilby et al., 1999). Heifers receiving GST had greater ($P < 0.01$) plasma glucose but similar ($P = 0.76$) plasma insulin concentrations compared with SAL heifers (Table 3). In the present study, the increase in plasma glucose concentrations in BST heifers despite similar insulin concentrations can be attributed t decreased insulin sensitivity caused by sometribove zinc administration (Dunshea et al., 1995). The main goal of Exp. 3 was to determine if circulating IGF-I also modulates hepatic P4 catabolism and consequent P4 concentrations given that this hormone directly regulates hepatocytes activity (Jones and Clemmons, 1995). However, mean plasma P4 concentrations were similar ($P = 0.67$) between BST and SAL heifers (Table 3), suggesting that hepatic P4 catabolism in bovine females in adequate nutritional status is not directly regulated by circulating IGF-I.

**Table 3.** Plasma concentrations of glucose, insulin, IGF-I, and P4 in beef heifers receiving s.c. injection containing 250 mg sometribove zinc (BST) or saline (SAL) in Exp. 3.

| Item | BST | SAL | SEM | *P*-Value |
|---|---|---|---|---|
| Glucose, mg/dL | 73.00 | 69.60 | 1.60 | < 0.01 |
| Insulin, ng/mL | 1.44 | 1.65 | 0.51 | 0.76 |
| IGF-I, ng/mL | 248.00 | 143.00 | 6.00 | < 0.01 |
| P4, ng/mL | 3.07 | 3.13 | 0.15 | 0.67 |

## Conclusions

Results collectively suggest that the effects of insulin on hepatic P4 degradation and circulating P4 concentrations in bovine females in adequate nutritional status are dependent on circulating glucose, but not IGF-I. In addition, results reported herein indicate that nutritional alternatives to increase circulating concentrations of glucose and insulin may benefit reproductive function of females in adequate nutritional status by increasing circulating concentrations of P4.

## Literature Cited

Bilby et al. 1999. Theriogenology 51:1285.

Dunshea et al. 1995. J. Clin. Invest. 88:614.

Jones and Clemmons. 1995. Endocr. Rev. 16:3.

Kegley et al. 2000. J. Anim. Sci. 78:3117.

Lemley et al. 2008. J. Dairy Sci. 91:641.

Lopes et al. 2009. J. Anim. Sci. 87:3935.

Mass. 1987. Vet. Clin. N. Amer. Food Anim. Pract. 3:633.

Nelson and Cox. 2005. Lehninger principles of biochemistry.

Pescara et al. 2010. Arq. Bras. Med. Vet. Zootec. 62:130.

Schillo et al. 1992. J. Anim. Sci. 70:3394.

Spicer and Echternkamp. 1995. Domest. Anim. Endocrinol. 12:223.

Vieira et al. 2010. J. Dairy Sci. 93:3407.

Wettemann et al. 2003. J. Anim. Sci. 81:E48.

Wiltbank et al. 1962. J. Anim. Sci. 21:219.

Oregon State University

BEEF086

Beef Research Report

Beef Cattle Sciences

# Effects of 24-h Transport or 24-h Nutrient Restriction on Acute-phase and Performance Responses of Feeder Cattle[1]

R. Marques[2], R. F. Cooke[2], C. Francisco[2,3], F. T. Cooke[2], T. A. Guarnieri Filho[3], and D. W. Bohnert[2]

## Synopsis

Feed and water restriction are major causes for the acute-phase reaction and reduced feedlot receiving performance typically detected in transported feeder cattle.

## Summary

The objective was to compare acute-phase and performance responses of weaned beef cattle exposed to transport or nutrient restriction. Angus × Hereford steers (n = 30) and heifers (n = 15) were balanced by sex and BW, and randomly assigned to 15 pens on d -12 of the experiment. On d 0, pens were randomly assigned to 1 of 3 treatments: 1) transport for 24 h in a livestock trailer (TRANS); 2) no transport, but feed and water deprivation for 24 h (REST); or 3) no transport and full access to feed and water (CON). Treatments were concurrently applied from d 0 to d 1. Total DMI was evaluated daily from d 1 to d 28. Full BW was recorded prior to treatment application and at the end of experiment. Blood samples were collected on d 0, 1, 4, 7, 10, 14, 21, and 28. Mean ADG was greater ($P < 0.01$) in CON vs. TRANS and REST cattle, but similar ($P = 0.46$) between TRANS and REST cattle. No treatment effects were detected on DMI, but CON had greater G:F vs. TRANS ($P < 0.01$) and REST cattle ($P = 0.08$), whereas G:F was similar ($P = 0.21$) between TRANS and REST cattle. Plasma cortisol concentrations were greater ($P \leq 0.05$) in REST vs. CON and TRANS cattle on d 1, 4, 7, 14, 21, and 28, and tended to be greater ($P = 0.10$) in TRANS vs. CON cattle on d 1. Serum NEFA was greater ($P < 0.01$) in REST and TRANS vs. CON cattle on d 1, but also greater ($P < 0.01$) in REST vs. TRANS cattle on d 1. Plasma ceruloplasmin peaked on d 4 for TRANS and REST cattle (day effects; $P < 0.01$) but did not change ($P = 0.58$) for CON cattle. Hence, CON cattle had reduced mean plasma ceruloplasmin concentration vs. TRANS ($P = 0.07$) and REST ($P = 0.01$) cattle. Plasma haptoglobin peaked on d 1 for TRANS and increased from d 1 to 14 in REST cattle (day effects; $P < 0.01$) but did not change ($P = 0.65$) for CON cattle. Hence, TRANS cattle had greater plasma haptoglobin vs. CON and REST cattle on d 1 ($P < 0.01$), whereas REST cattle had greater ($P \leq 0.05$) plasma haptoglobin vs. TRANS and CON cattle on d 7. In conclusion, 24-h transport and 24-h nutrient restriction elicited acute-phase protein reactions, and similarly reduced performance of feeder cattle.

## Introduction

Cattle are inevitably exposed to stress during their productive life (Carroll and Forsberg, 2007), including psychologic, physiologic, and physical

1. This document is part of the Oregon State University – 2012 Beef Research Report. Please visit the Beef Cattle Sciences website at http://beefcattle.ans.oregonstate.edu.
2. Oregon State University - Eastern Oregon Agricultural Research Center, Burns, OR.
3. UNESP – Universidade Estadual Paulista, FMVZ/DPA, Botucatu, São Paulo, Brazil.

stressors associated with management procedures currently practiced within beef and dairy production systems. An example is road transport, one of the most stressful events in the productive life of a feeder calf. Upon long transportation periods feeder cattle experience inflammatory and acute-phase responses that often lead to impaired health and productivity during feedlot receiving (Berry et al., 2004; Araujo et al., 2010; Cooke et al., 2011). These stress-induced immune responses may be elicited by several stressors that cattle are exposed to during road transport, including feed and water restriction. In fact, preliminary data from our research group indicated that water and feed deprivation for 24 h increased circulating concentrations of acute-phase proteins in overtly healthy beef steers (Cappellozza et al., 2011).

Therefore, we hypothesized that feed and water restrictions are major stimulants of the acute-phase response elicited by road transport. Based on our hypothesis, the objective of this experiment was to compare the effects of 24-h road transport or 24-h water and feed restriction on acute-phase and feedlot receiving performance responses of feeder cattle.

## Materials and Methods

This experiment was conducted at the Eastern Oregon Agricultural Research Center, Burns in accordance with an approved Oregon State University Animal Care and Use protocol. Forty-five Angus x Hereford steers (n = 30) and heifers (n = 15) weaned at 7 mo of age were ranked by sex and initial BW (217 ± 3 kg) on d -12 of the study, and randomly allocated to 15 dry lot pens (3 animals/pen; 2 steers and 1 heifer). From d -12 to 0, all pens received alfalfa-mixed hay for ad libitum consumption and 2.3 kg/hd daily (DM basis) of a supplement containing (as-fed basis) 84% corn, 14% soybean meal, and 2% mineral mix. On d 0, pens were assigned to 1 of 3 treatments: 1) transport for 24 h in a commercial livestock trailer for approximately 1,200 km (TRANS), 2) no transport, but feed and water deprivation for 24 h (REST), or 3) no transport and full access to feed and water (CON). Treatments were concurrently applied from d 0 to d 1. On d 1, TRANS and REST cattle returned to their original pens, and all pens received the same diet offered prior to treatment application.

Total and forage DMI were evaluated daily from d 1 to 28. Full BW was recorded prior to (d -1 and 0) treatment application and at the end of experiment (d 28 and 29) for ADG calculation. Total gain and DMI from d 1 to 28 were used for G:F calculation. Blood samples were collected on d 0 (prior to treatment application), 1 (immediately at the end of treatments), 4, 7, 10, 14, 21, and 28, via jugular venipuncture into commercial blood collection tubes (Vacutainer, 10 mL; Becton Dickinson, Franklin Lakes, NJ) containing or not sodium heparin for serum and plasma collection, respectively. Plasma samples were analyzed for concentrations of cortisol (Endocrine Technologies Inc., Newark, CA), haptoglobin (Cooke and Arthington, 2012), and ceruloplasmin (Demetriou et al., 1974). Serum samples were analyzed for concentrations of NEFA (Wako Chemicals: Dallas, TX). Data were analyzed using the PROC MIXED procedure of SAS (SAS Inst., Inc., Cary, NC) and Satterthwaite approximation to determine the denominator df for the tests of fixed effects. The model statement used for BW shrink from d 0 to d 1 and ADG contained the effects of treatment, sex, and the interaction. Data were analyzed using calf(treatment × pen) as random variable. The model statement used for DMI and G:F contained the effects of treatment, as well as day and the resultant interaction for DMI only. Data were analyzed using pen(treatment) as the random variable. The model statement used for hormones and metabolites contained the effects of treatment, day, sex, and the resultant interactions. Data were analyzed using calf(treatment × pen) as the random variable. The specified term for repeated statements was day, pen(treatment) or calf(treatment × pen) as subject for DMI or hormones and metabolites, respectively, and the covariance structure utilized was based on the Akaike information criterion. Results are reported as least square means and were separated using PDIFF. Significance was set at $P \leq 0.05$. Results are reported according to treatment effects if no interactions were significant, or according to the highest-order interaction detected.

## Results and Discussion

Body weight shrink from d 0 to d 1 was similar ($P = 0.16$) between TRANS and REST, and greater ($P < 0.01$) for both treatments vs. CON (Table 1). Mean ADG was greater ($P < 0.01$) in CON vs. TRANS and REST cattle, and similar ($P = 0.46$) between TRANS and REST cattle (Table 1). No treatment ($P \geq 0.25$) effects were detected on forage, concentrate, and total DMI (Table 1). However, CON had greater G:F vs. TRANS ($P < 0.01$) and tended to have greater G:F vs. REST cattle

($P = 0.08$), whereas G:F was similar ($P = 0.21$) between TRANS and REST cattle (Table 1). Similar to previous research, road transport reduced ADG and G:F during feedlot receiving (Cole et al., 1988). Further, REST cattle experienced similar feedlot receiving performance compared with TRANS cohorts, suggesting that feed and water deprivation are major causes for the reduced performance of transported cattle.

**Table 1.** Feedlot receiving performance of cattle submitted to transport for 24 h for approximately 1,200 km (TRANS), no transport but feed and water deprivation for 24 h (REST), or no transport and full access to feed and water (CON). [1]

| Item | CON | REST | TRANS | SEM | *P* = |
|---|---|---|---|---|---|
| DMI, kg/d | | | | | |
| Forage | 5.5 | 4.9 | 5.4 | 0.3 | 0.32 |
| Concentrate | 2.3 | 2.3 | 2.3 | 0.1 | 0.52 |
| Total | 7.9 | 7.2 | 7.8 | 0.4 | 0.25 |
| ADG, [2] kg/d | 1.27[a] | 0.97[b] | 0.91[b] | 0.05 | < 0.01 |
| G:F, [3] g/kg | 163[a] | 143[ab] | 127[b] | 7 | 0.03 |
| Shrink, [4] % | 0.07[a] | 8.1[b] | 9.6[b] | 0.7 | < 0.01 |

[1] Within rows, values with different superscripts differ ($P < 0.05$).
[2] Calculated using full BW values obtained prior to (d -1 and 0) treatment application and at the end of experiment (d 28 and 29).
[3] Calculated using total DMI and BW gain from d 0 to d 28.
[4] Based on BW loss from d 1 relative to d 0.

Treatment × day interactions were detected ($P < 0.05$) for cortisol, NEFA, haptoglobin, and ceruloplasmin. Plasma cortisol concentrations were greater ($P < 0.05$) in REST compared to CON and TRANS cattle on d 1, 4, 7, 14, 21, and 28, and tended to be greater ($P = 0.10$) in TRANS compared to CON cattle on d 1 (Figure 1). Serum NEFA concentrations were greater ($P < 0.01$) in REST and TRANS compared to CON cattle on d 1, but also greater ($P < 0.01$) in REST compared to TRANS cattle on d 1 (Figure 1). Plasma ceruloplasmin concentrations peaked on d 4 for TRANS and REST cattle (day effects; $P < 0.01$) but did not change ($P = 0.58$) for CON cattle (Figure 2). Hence, CON cattle had reduced mean plasma ceruloplasmin concentration compared to TRANS ($P = 0.07$) and REST ($P = 0.01$) cattle. Plasma haptoglobin peaked on d 1 for TRANS and increased from d 1 to 14 in REST cattle (day effects; $P < 0.01$) but did not change ($P = 0.65$) for CON cattle (Figure 2). Hence, TRANS cattle had greater plasma haptoglobin compared to CON and REST cattle on d 1 ($P < 0.01$), whereas REST cattle had greater ($P \leq 0.05$) plasma haptoglobin compared to TRANS and CON cattle on d 7 (Figure 2).

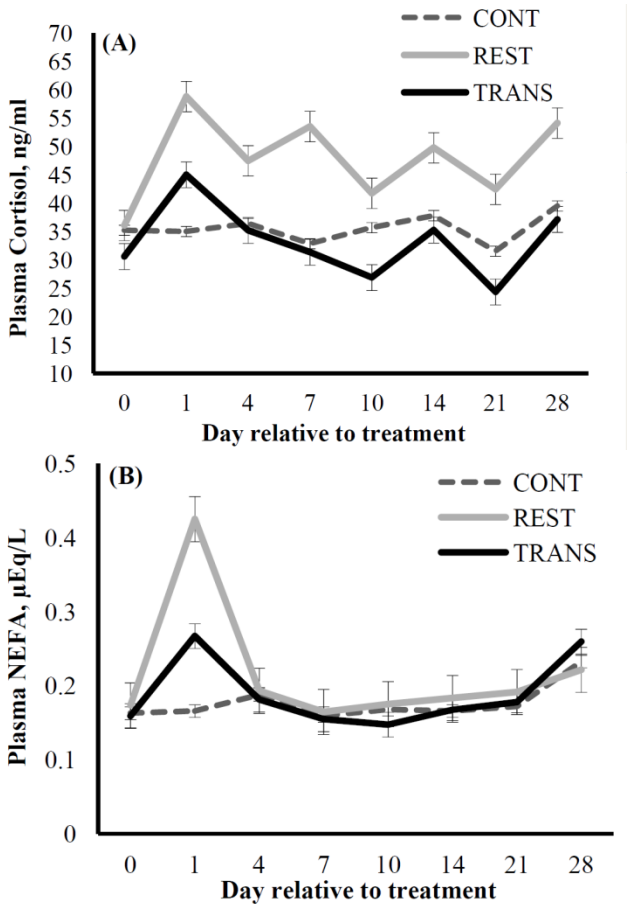


**Figure 1.** Plasma cortisol (Panel A) and serum NEFA (Panel B) in cattle submitted to transport for 24 h for approximately 1,200 km (TRANS), no transport but feed and water deprivation for 24 h (REST), or no transport and full access to feed and water (CON). Treatment × day interactions were detected ($P < 0.05$).

These results suggest that TRANS and REST stimulated mobilization of body reserves, elicited a neuroendocrine stress response, and induced an acute-phase protein reaction that impaired feedlot receiving ADG and G:F (Ellenberger et al., 1989; Sapolsky, 2000; Carroll and Forsberg, 2007). Previous research also reported increased circulating cortisol, ceruloplasmin, and haptoglobin in feeder cattle following road transport, and attributed these outcomes to impaired feedlot receiving performance (Crookshank et al., 1979; Araujo et al., 2010; Cooke et al., 2011). Conversely, the specific effects of feed and water restriction on neuroendocrine and acute-phase parameters have not yet been determined. Supporting these outcomes, recent research from our group demonstrated that

neuroendocrine stress reactions can stimulate breakdown of body reserves and activate acute-phase and inflammatory processes in bovine (Cooke et al., 2012). In addition, feed and water deprivation may result in death of rumen microbes and subsequent release of endotoxins (Meiske et al., 1958), which may be absorbed by the ruminal wall and small intestine, incorporated into the circulation (Chin et al., 2006), and elicit neuroendocrine and acute-phase reactions (Carroll et al., 2009). Hence, the acute-phase protein reaction detected in TRANS and REST cattle can be attributed to the increase in circulating cortisol, NEFA, and altered ruminal flora following treatment application.

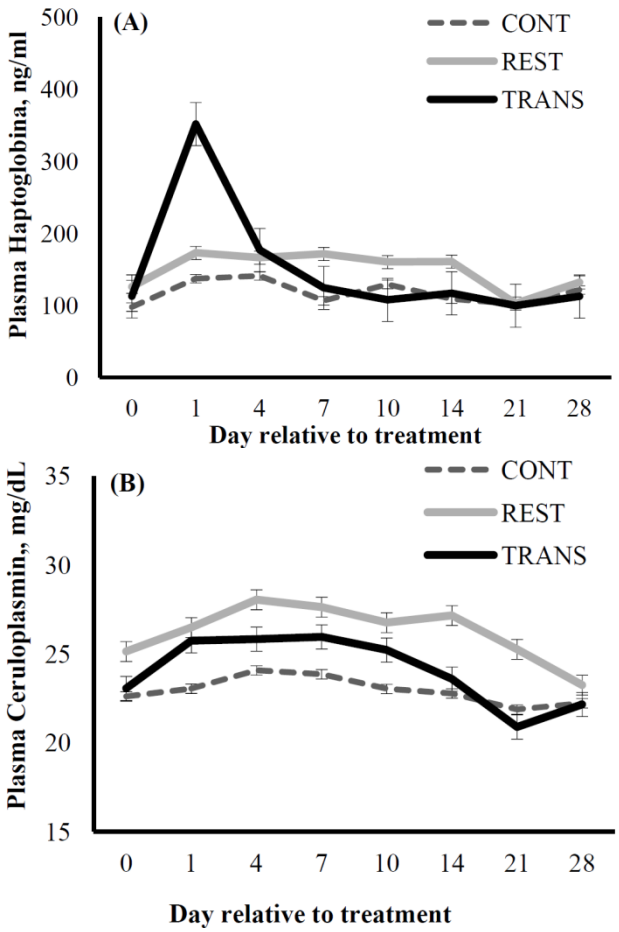


**Figure 2.** Plasma haptoglobin (Panel A) and ceruloplasmin (Panel B) in cattle submitted to transport for 24 h for approximately 1,200 km (TRANS), no transport but feed and water deprivation for 24 h (REST), or no transport and full access to feed and water (CON). Treatment × day interactions were detected ($P < 0.05$).

It is also important to note that the increase in circulating NEFA, cortisol, and ceruloplasmin concentrations was more severe in REST vs. TRANS cattle. Similarly, circulating haptoglobin remained elevated for a longer period in REST vs. TRANS cattle. These results suggest that neuroendocrine stress response was more severe in REST cattle, which caused or was caused by the greater mobilization of body tissues, and resulted in the greater acute-phase reaction compared with that observed in TRANS cohorts. The reasons for this outcome are unknown and deserve further investigation, particularly because TRANS steers also experienced a 24-h feed and water restriction during transport.

## Conclusions

In conclusion, 24-h transport and 24-h nutrient restriction elicited acute-phase protein responses and similarly reduced performance of feeder cattle. Therefore, feed and water restriction are major causes for the acute-phase reaction and reduced feedlot receiving performance typically detected in transported feeder cattle.

## Literature Cited

Araujo et al. 2010. J. Anim. Sci. 87:4125-4132.

Arthington et al. 2008. J. Anim. Sci. 86:2016-2023.

Berry et al. 2004. J. Anim. Sci. 82:845–850.

Cappellozza et al. 2011. J. Anim. Sci. 89:269.

Carroll and Forsberg. 2007. Vet. Clin. Food. Anim. 23:105-149.

Carroll et al. 2009. J. Anim. Sci. 87:4167-4172.

Chin et al. 2006. Can. J. Physiol. Pharmacol. 84:1043–1050.

Cole et al. 1988. Am. J. Vet. Res. 49:178–183.

Cooke and Arthington. 2012. J. Anim. Physiol. Anim. Nutr. doi: 10.1111/j.1439-0396.2012.01298.

Cooke et al. 2012. J. Anim. Sci. doi:10.2527/jas. 2011- 4608.

Cooke et al. 2011. J. Anim. Sci. 89:3677-3689.

Crookshank et al. 1979. J. Anim. Sci. 48:430-435.

Demetriou et al. 1974. Clinical Chemistry: Principles and Techniques.

Ellenberger et al. 1989. J. Anim. Sci. 67:1446

Meiske et al. 1958. J Anim Sci 17, 774– 781.

Sapolsky et al. 2000. Endocr. Rev. 21:55-89.

Oregon State University

BEEF087

Beef Cattle Sciences

Beef Research Report

# Influence of the Amount and Supplementation Frequency of Protein on Utilization of Low-Quality Forage by Ruminants[1]

D. W. Bohnert[2], R. F. Cooke[2], S. J. Falck[2], B. I. Cappellozza[2], M. Van Emon[3], and C. S. Schauer[3]

## Synopsis

When providing supplemental CP to ruminants consuming low-quality forage at extended intervals, such as once every 10 days, it is possible for managers to maintain acceptable forage intake, digestibility of nutrients, and cow performance by reducing the amount, and cost, of supplement provided.

## Summary

Three experiments were conducted to evaluate the effect of amount and frequency of crude protein (CP) supplementation on ruminants consuming low-quality forage. Treatments were arranged in a 2 × 3 factorial design (two levels of CP provided daily, once every 5 days, or once every 10 days) with an unsupplemented control. The greater level of CP was estimated to meet ruminal requirements for degradable intake protein and the lower level was 50% of the greater level. Soybean meal (SBM) was used as the CP supplement. Seven steers (661 ± 20 lb; Experiment 1) and 7 wethers (68 ± 1 lb; Experiment 2) were used in duplicate 4 × 7 incomplete Latin square designed experiments to determine the influence of treatments on nutrient intake and digestion. Experimental periods were 30 days with feed and digesta collected on d 19 through 28 and day 21 through 30, respectively, for estimation of nutrient digestibility.

Eighty-four cows (1,231 ± 9 lb; 4.8 ± 0.04 body condition score; BCS) in the last third of gestation were used in Experiment 3 to evaluate treatment effects on weight and body condition score (BCS) change. Treatments were evaluated using the following contrasts: 1) Control vs CP supplementation, 2) Full CP vs Half CP, 3) linear effect of supplementation frequency, 4) quadratic effect of supplementation frequency, 5) Interaction of linear effect of supplementation frequency and level of CP, and 6) Interaction of quadratic effect of supplementation frequency and level of CP.

Hay intake by steers increased ($P = 0.03$) with CP supplementation but only tended to increase ($P = 0.08$) with Full CP compared with Half CP. In contrast, hay and total intake by lambs was not affected ($P > 0.25$) by CP supplementation. Interestingly, a linear effect of CP amount × supplementation frequency interaction for both hay and total intake was noted for steers ($P = 0.02$) and a tendency was noted for lambs ($P < 0.09$), with intake decreasing a greater amount from daily to once every10 days with Full CP supplementation compared with little to no reduction with Half CP.

Diet digestibility by steers tended ($P = 0.10$) to be greater with CP supplementation and was increased ($P < 0.01$) by lambs. This, with the intake data, resulted in a greater quantity of nutrients

1. This document is part of the Oregon State University – 2012 Beef Research Report. Please visit the Beef Cattle Sciences website at http://beefcattle.ans.oregonstate.edu.
2. EOARC – Oregon State University and USDA – Agricultural Research Center, Burns 97720.
3. North Dakota State University – Hettinger Research and Extension Center, Hettinger, ND 58639.

available for utilization by the animal with CP supplementation.

Efficiency of CP utilization by lambs was greater with CP supplementation but was not altered by amount of supplement ($P = 0.94$) or supplementation frequency ($P > 0.92$). In addition, plasma urea was greater with CP supplementation ($P < 0.01$) and for Full CP compared with Half CP ($P \leq 0.02$) in both steers and lambs.

Cow pre- and post-calving weight and BCS change was improved with CP supplementation ($P \leq 0.03$). Likewise, pre- and post-calving weight change and pre-calving BCS change were improved ($P \leq 0.01$) with Full CP compared with Half CP. However, the change in pre-calving weight and BCS was less as supplementation frequency decreased for Half CP compared with Full CP ($P = 0.01$).

These data suggest that reducing the amount of supplemental CP, when supplementation intervals are greater than 5 or 6 days, can be a management tool to maintain acceptable levels of intake, digestibility, and cow performance while reducing supplement cost.

## Introduction

Production of beef cattle is consistently the number two agriculture commodity in Oregon. Consequently, raising cattle is the largest generator of livestock value in Oregon and is dominated by commercial cow/calf production with over 500,000 producing females located in the state. Most cattle spend their entire lives, except for the final 4 to 6 months in the feedlot, grazing standing forage or consuming hay. Forage quality is usually sufficient to support normal levels of production early in the growing season; however, as forages mature they increase in fiber content, decrease in CP, and decrease in digestibility. As a result, many cattle in Oregon and the western United States consume low-quality forage (< 6% CP) from late summer through winter and require some form of supplementation to maintain desired levels of performance.

Protein supplementation of low-quality forage has been shown to increase cow weight gain and BCS, forage intake and digestibility, and can improve reproductive performance. However, winter supplementation can be very expensive. Winter feed costs in the intermountain west often total $150 to 250 per cow per year. In addition to actual supplement costs, winter supplementation includes other expenses such as the labor, time, and equipment associated with supplement delivery. In contrast to other areas of North America, winter feed costs represent an economic disadvantage and could substantially threaten the economic future of the beef industry in this region.

Decreasing the frequency of protein supplementation is one management practice that can decrease labor and time costs by greater than 80% compared with daily supplementation. Ruminants have the ability to recycle excess absorbed nitrogen back to the rumen; therefore, recycling of absorbed nitrogen may support ruminal fermentation between times of supplementation. Consequently, research has shown that protein supplements can be fed at infrequent intervals and still maintain acceptable levels of performance (Hunt et al., 1989; Huston et al., 1999; Bohnert et al., 2002); however, data is limited comparing the effects of altering the amount of protein provided at infrequent intervals on forage intake and digestibility, animal performance, and efficiency of protein use.

It is possible that ruminants consuming low-quality forage may be able to adapt to infrequent supplementation of CP by increasing their ability to recycle nitrogen, thereby improving efficiency of CP use. We hypothesize that as the supplementation interval increases ruminants will become more efficient in their use of supplemental CP. As a result, we should be able to provide LESS total CP and maintain performance comparable to more frequent supplementation of MORE total CP. This will not only save time and labor, but will decrease the amount and cost of supplement provided to beef cows consuming low-quality forage, and therefore increase economic returns of Oregon's beef producers (Table 1).

## Materials and Methods

*Experiment 1.* Seven ruminally cannulated Angus x Hereford steers (661 ± 22 lb) were used in a 4 × 7 incomplete Latin square design and housed in individual pens within an enclosed barn with continuous lighting. Steers were provided continuous access to fresh water and a low-quality cool season hay (Chewings fescue grass seed straw; 2.9% CP). A trace mineralized salt mix was provided daily. Treatments were arranged in a 2 × 3 factorial design with 2 levels of CP provided daily, once every 5 days, or once every 10 days with an unsupplemented control (daily, 5-day, and 10-day treatments, within CP level, received the same total amount of CP over a 10-day period). The greater level of CP was estimated to meet ruminal requirements for degradable intake protein and the lower level was 50% of the greater level. Soybean

meal (SBM; 51.4% CP) was placed directly into the rumen via the ruminal cannula for supplemented treatments.

Experimental periods were 30 d, with intake measured beginning d 19 and concluding d 28. On day 11 (day of supplementation for all treatments except for control) and day 20 (day before supplementation for all treatments except for control), treatment effects on ruminal indigestible fiber fill were determined by manually removing the contents from each steer's reticulo-rumen 4 h after feeding. Feces were collected on days 21 to 30.

On days 21 and 30, ruminal fluid was collected by suction strainer immediately prior to feeding and at 3, 6, 9, 12, 18, and 24 hours post-feeding. Ruminal fluid pH was measured immediately after collection.

Data were analyzed as an incomplete 7 × 4 Latin square. The model for intake and digestibility data included period and treatment. The model for samples collected at fixed times included period, treatment, time, and treatment × time. Contrast statements were: 1) Control vs CP supplementation, 2) Full vs Half CP, 3) linear effect of supplementation frequency, 4) quadratic effect of supplementation frequency, 5) Interaction of linear effect of supplementation frequency and level of CP, and 6) Interaction of quadratic effect of supplementation frequency and level of CP.

*Experiment 2.* Seven wethers (68 ± 1 lb) were used in a 4 × 7 incomplete Latin square design. Lambs were provided continuous access to fresh water and a low-quality cool season hay (Chewings fescue grass seed straw; 4.9% CP). A trace mineralized salt mix was provided daily. Treatments were arranged in a 2 × 3 factorial design (two levels of CP provided daily, once every 5 days, or once every 10 days) with an unsupplemented control. The greater level of CP was estimated to meet the CP requirement of a 66 lb lamb gaining 0.44 lb/day; the lower level was 50% of the greater level. Soybean meal (SBM; 49.9% CP) was used as the CP supplement and was offered to lambs immediately prior to hay feeding.

Experimental periods were 30 d, with intake measured beginning d 19 and concluding d 28. Feces and urine were collected on days 21 to 30. In addition, blood samples were collected on days 21 to 30 for analysis of plasma urea.

Data were analyzed as an incomplete 7 × 4 Latin square. The model for intake and digestibility data included period and treatment. The model for plasma urea included period, treatment, day, and treatment × day. The same contrasts described in Experiment 1 were used to evaluate treatment effects.

*Experiment 3.* Eighty-four cows (1231 ± 9 lb; 4.8 ± 0.04 BCS) in the last third of gestation were stratified by age, body condition score, and weight and assigned randomly within stratification to the treatments described in Experiment 1 using a Randomized Complete Block design. Soybean meal was used as the source of supplemental CP (51.7% CP). The cows were then sorted by treatment and allotted randomly to 1 of 21 pens. The greater level of CP was, on a daily basis, 0.525 lb CP/hd and the lower level was 50% of the greater level. Supplements were provided through calving. Cows had continuous access to water, salt, and a vitamin/mineral mix. They were offered ad libitum access to low-quality grass seed straw (2.4% CP) at 0800 daily.

Cow weight and BCS were measured every 14 days until calving and within 24 hours after calving. In addition, calf weights were obtained within 24 hours of birth.

Data were analyzed as a Randomized Complete Block. The model included block, treatment, and Block × treatment. The same contrasts described in Experiment 1 were used to evaluate treatment effects.

## Results

*Experiment 1.* Hay ($P = 0.03$) and total ($P < 0.01$) intake increased with CP supplementation; however, we noted a linear effect of CP amount × supplementation frequency interaction ($P = 0.02$) for both hay and total intake, with intake decreasing almost 17% from daily to once every10 days with Full CP supplementation compared with essentially no reduction with Half CP (Table 2). Digestibility was not altered by CP supplementation ($P = 0.10$) but it increased quadratically ($P < 0.01$) as the supplementation interval increased. Fiber digestibility (neutral detergent fiber) was not affected by treatments ($P > 0.12$).

Ruminal particulate fill was not affected by treatments on the day all supplements were provided ($P > 0.31$; Table 3); however, when only daily supplements were provided, ruminal particulate fill was greater ($P = 0.03$) with CP supplementation. Also, ruminal particulate passage rate was increased with CP supplementation ($P > 0.03$).

A day × treatment interaction ($P < 0.01$) was noted for plasma urea (Figure 1); however, after

evaluating the nature of the responses we decided to provide the day × treatment figure and discuss overall treatment means. Plasma urea increased with CP supplementation ($P < 0.01$; Table 2) and was greater with Full CP compared with Half CP ($P < 0.01$).

Ruminal pH decreased linearly as supplementation frequency decreased ($P < 0.01$) when all supplements were provided; however no affect was noted when only daily supplements were provided ($P > 0.22$).

A time × treatment interaction ($P < 0.01$) was noted for ruminal ammonia when all supplements were provided (Figure 2); however, after evaluating the nature of the responses we decided to provide the time × treatment figure and discuss overall treatment means. Ruminal ammonia increased with CP supplementation when all supplements were provided and was greater with Full CP compared with Half CP ($P < 0.01$). However, a linear effect of CP amount × supplementation frequency interaction ($P = 0.02$) was observed with ruminal ammonia increasing 400% from daily to once every10 days with Full CP supplementation compared with approximately 300% with Half CP (Table 3; Figure 2). When only daily supplements were provided, we noted no CP supplementation effect ($P = .44$) or difference between Full CP and Half CP ($P = .64$); nevertheless, ruminal ammonia decreased as supplementation frequency decreased ($P < 0.01$).

*Experiment 2.* Hay and total intake were not affected ($P > 0.25$) by CP supplementation. However, similar to Experiment 1, a tendency for a linear effect of CP amount × supplementation frequency interaction ($P \leq 0.09$) was noted for both hay and total intake, with intake decreasing over 30% from daily to once every10 days with Full CP supplementation compared with less than 10% with Half CP (Table 4).

Digestibility was increased 19% with CP supplementation ($P < 0.01$) and also increased ($P = 0.04$) as the supplementation interval increased. No difference in digestibility was noted between Full CP and Half CP ($P = 0.28$). As with intake, fiber digestibility (neutral detergent fiber) was increased ($P = 0.02$) almost 10% with CP supplementation. Also, fiber digestibility increased 11% as supplementation frequency decreased from daily to once every 10 days with Full CP compared with a 3% decrease with Half CP ($P = 0.04$).

Crude protein intake increased with CP supplementation ($P < 0.01$), for Full CP compared with Half CP ($P < 0.01$), and decreased as supplementation interval increased ($P = 0.04$). Digestibility of CP was increased greater than 300% with CP supplementation ($P < 0.01$), 21% greater for Full CP compared with Half CP ($P < 0.01$), and decreased as supplementation interval increased ($P = 0.01$).

The efficiency of CP use, measured as the quantity of digested CP retained in the body, was increased with CP supplementation ($P < 0.01$) but was not affected by amount of supplemental CP ($P = 0.94$) or supplementation frequency ($P > 0.92$) (Table 4).

As with Experiment 1, a day × treatment interaction ($P < 0.01$) was noted for plasma urea (Figure 3); however, after evaluating the nature of the responses we decided to provide the day × treatment figure and discuss overall treatment means. Plasma urea increased with CP supplementation ($P < 0.01$; Table 4) and was greater with Full CP compared with Half CP ($P = 0.03$).

*Experiment 3.* Pre- and Post-calving weight change by cows was improved with CP supplementation ($P < 0.03$) and for Full CP compared with Half CP ($P < 0.02$; Table 5). However, both pre- and post-calving weight change were negatively affected as supplementation frequency decreased ($P < 0.01$). It is of interest to note that there was less pre-calving weight change as supplementation frequency decreased from daily to once every 10 days for Half CP compared with Full CP ($P = 0.01$). Calf birth weight was not affected by treatment ($P > 0.19$).

Similar to our observations with cow body weight, pre- and post-calving change in BCS was improved with CP supplementation ($P < 0.03$). Also, pre-calving BCS change was improved with Full CP compared with Half CP ($P < 0.01$; Table 5) but negatively affected as supplementation frequency decreased ($P = 0.02$). Also, as with cow weight change, there was less pre-calving BCS change as supplementation frequency decreased for Half CP compared with Full CP ($P = 0.05$).

## Conclusions

Reducing the amount of supplemental CP provided to ruminants consuming low-quality forages, when supplementation intervals are greater than 5 or 6 days, can be a management tool to maintain acceptable levels of intake, digestibility, and cow performance while reducing supplement cost.

## Acknowledgements

This research study was funded by the Oregon Beef Council and the Agricultural Research Foundation. We would like to thank Lynn Carlon, Tony Runnels, Aaron Kennedy, Skip Nyman, Maria Reis, Felipe Sanches, and Flavia Cooke for their assistance in this study.

## Literature Cited

Bohnert et al. 2002. J. Anim. Sci. 80:1629-1637.

Hunt et al. 1989. J. Anim. Sci. 67:1360-1366.

Huston et al. 1999. J. Anim. Sci.77:3057-3067

**Figure 1.** Effect of protein amount and supplementation frequency on plasma urea nitrogen in steers. Columns from left to right for each treatment represent day 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 of a 10-day supplementation period, respectively. Treatments were: Control; D = 0.133% of body weight/day of soybean meal (SBM); 5D = 0.665% of body weight of SBM once every 5 days; 10D = 1.33% of body weight of SBM once every 10 days; 50% D = 50% of the D treatment; 50% 5D = 50% of the 5D treatment; 50% 10D = 50% of the 10D treatment. Each column with an S below it represents a supplementation day.

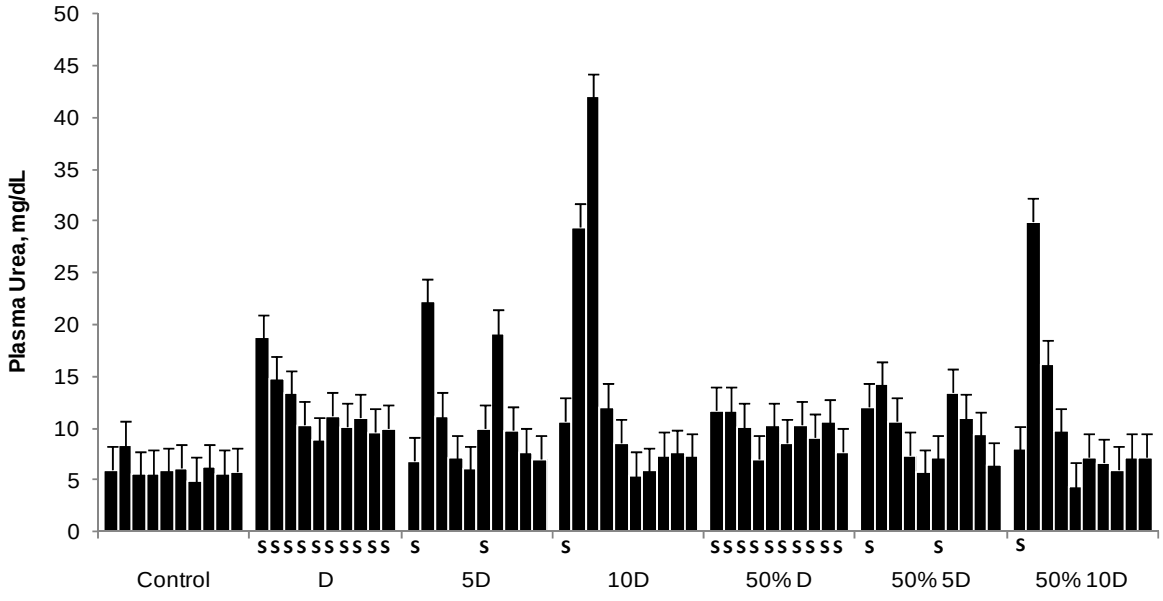

**Figure 2**. Effect of protein amount and supplementation frequency on steer ruminal ammonia N the day all supplements were provided. Columns from left to right for each treatment represent 0, 3, 6, 9, 12, 18, and 24 hours post-feeding, respectively. Treatments were: Control; D = 0.133% of body weight/day of soybean meal (SBM); 5D = 0.665% of body weight of SBM once every 5 days; 10D = 1.33% of body weight of SBM once every 10 days; 50% D = 50% of the D treatment; 50% 5D = 50% of the 5D treatment; 50% 10D = 50% of the 10D treatment.

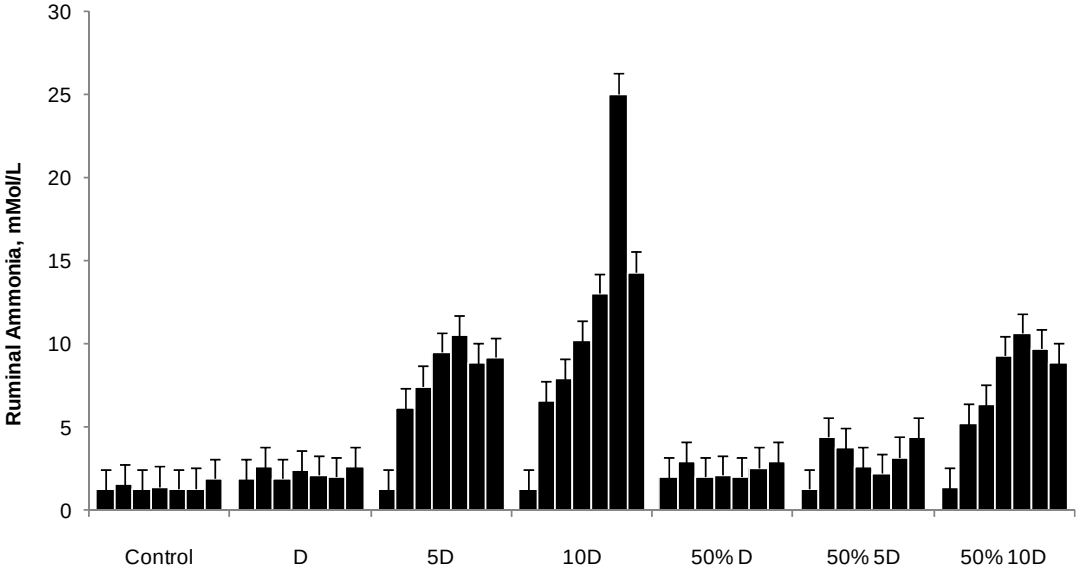


**Figure 3**. Effect of protein amount and supplementation frequency on plasma urea nitrogen in lambs. Columns from left to right for each treatment represent day 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 of a 10-day supplementation period, respectively. Treatments were: Control; D = 0.133% of body weight/day of soybean meal (SBM); 5D = 0.665% of body weight of SBM once every 5 days; 10D = 1.33% of body weight of SBM once every 10 days; 50% D = 50% of the D treatment; 50% 5D = 50% of the 5D treatment; 50% 10D = 50% of the 10D treatment. Each column with an S below it represents a supplementation day.

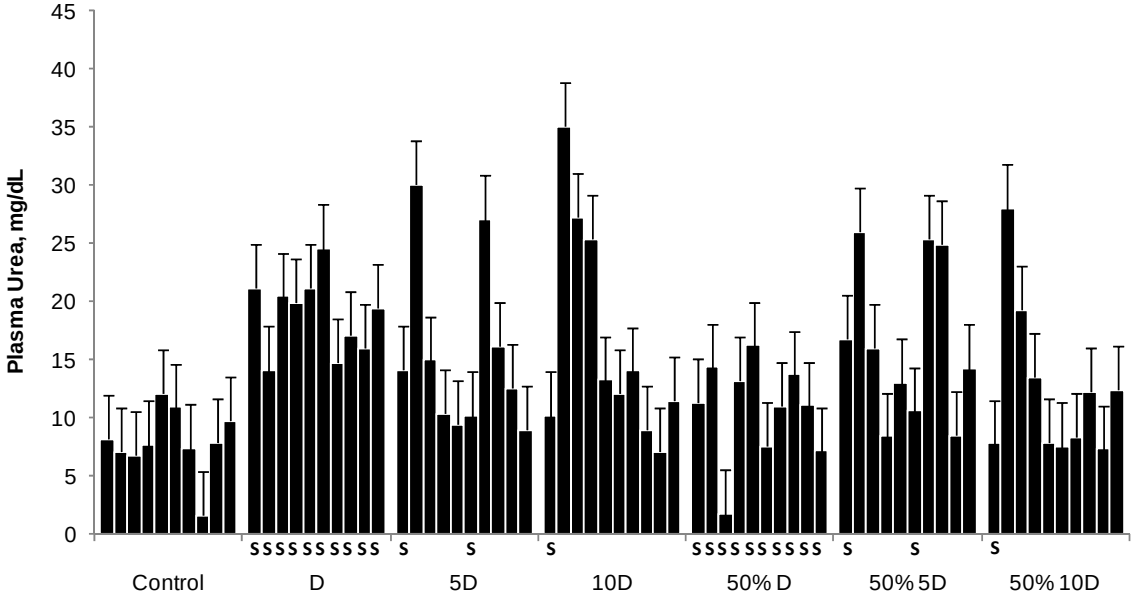

**Table 1.** Estimated cost of treatments over a 30-day Period. One pound of soybean meal (SBM), daily, was used as the basis to compare all other treatments.

| | 1 pound of Soybean Meal/head | | | 1/2 pound of Soybean Meal/head | | |
|---|---|---|---|---|---|---|
| | Daily | 5 Days | 10 Days | Daily | 5 Days | 10 Days |
| Fuel Cost ($)[a] | 360.00 | 72.00 | 36.00 | 360.00 | 72.00 | 36.00 |
| Labor Cost($)[b] | 630.00 | 126.00 | 63.00 | 630.00 | 126.00 | 63.00 |
| Supplement Cost ($)[c] | 1,485.00 | 1,485.00 | 1,485.00 | 742.50 | 742.50 | 742.50 |
| Total Cost ($) | 2,475.00 | 1,683.00 | 1,584.00 | 1732.50 | 940.50 | 841.50 |
| | | | | | | |
| Labor/Fuel Cost Reduction | 0 | 80% | 90% | 0 | 80% | 90% |
| Supplement Cost Reduction | 0 | 0 | 0 | 50% | 50% | 50% |
| Total Cost Reduction | 0 | 32% | 36% | 30% | 62% | 66% |
| Total Benefit ($) | 0 | 792.00 | 891.00 | 742.50 | 1,534.50 | 1633.50 |

[a] Fuel costs calculated as 3 gallons/supplementation day at $4.00/gallon
[b] Labor calculated as 2.5 hours/supplementation day at $8.40/hour
[c] Assuming 300 cow herd; cost of $330/ton

Page 25

**Table 2.** Effect of CP amount (soybean meal; SBM) and supplementation frequency on intake, diet digestibility, and plasma urea in steers.

| | Treatment[a] | | | | | | | | P-Value[c] | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Con | D | 5D | 10D | 50% D | 50% 5D | 50% 10D | SEM[b] | Con vs Supp | Full vs Half | L Freq | Q Freq | L Freq vs Amt | Q Freq vs Amt |
| Hay intake, % body weight | 1.61 | 1.96 | 1.92 | 1.62 | 1.76 | 1.70 | 1.75 | 0.075 | 0.03 | 0.08 | 0.02 | 0.52 | 0.02 | 0.14 |
| SBM intake, % body weight | 0.000 | 0.133 | 0.133 | 0.133 | 0.067 | 0.067 | 0.067 | | | | | | | |
| Total Intake, % body weight | 1.61 | 2.10 | 2.05 | 1.76 | 1.83 | 1.77 | 1.81 | 0.075 | < 0.01 | < 0.01 | 0.02 | 0.52 | 0.02 | 0.14 |
| Diet Digestibility, % | 45.0 | 45.8 | 44.7 | 48.6 | 45.7 | 45.1 | 47.3 | 0.86 | 0.10 | 0.57 | < 0.01 | < 0.01 | 0.39 | 0.34 |
| NDF Digestibility, % | 48.2 | 47.5 | 46.2 | 49.0 | 47.8 | 47.4 | 48.5 | 1.03 | 0.64 | 0.68 | 0.26 | 0.12 | 0.69 | 0.48 |
| Plasma Urea, mg/dL | 6.0 | 11.8 | 10.7 | 13.6 | 9.7 | 9.7 | 10.2 | 0.72 | <0.01 | <0.01 | 0.10 | 0.07 | 0.38 | 0.16 |

[a] CON = control; D = 0.133 % body weight/day of SBM; 5D = 0.665% body weight of SBM once every 5 days; 10D = 1.33% body weight of SBM once every 10 days; 50% D = 50% of the D treatment; 50% 5D = 50% of the 5D treatment; 50% 10D = 50% of the 10D treatment.
[b] n = 4.
[c] Con vs Supp = control vs supplemented treatments; Full vs Half = full vs half amount of CP; L Freq = linear effect of supplementation frequency; Q SF = quadratic effect of supplementation frequency; L Freq vs Amt = interaction of the linear effect of supplementation frequency and amount of CP; Q Freq vs Amt = interaction of the quadratic effect of supplementation frequency and amount of CP.

**Table 3.** Ruminal particulate fill and ammonia concentration on the day of supplementation for all supplemented treatments and, other than daily treatments, the day before supplementation in steers.

| | Treatment[a] | | | | | | | | P-value[c] | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Con | D | 5D | 10D | 50% D | 50% 5D | 50% 10D | SEM[b] | Con vs Supp | Full vs Half | L Freq | Q Freq | L Freq vs Amt | Q Freq vs Amt |
| ***Day of Supplementation*** | 1.61 | 1.96 | 1.92 | 1.62 | 1.76 | 1.70 | 1.75 | 0.075 | 0.03 | 0.08 | 0.02 | 0.52 | 0.02 | 0.14 |
| Particulate Fill, % BW | 0.90 | 1.02 | 1.07 | 0.99 | 0.95 | 0.89 | 0.89 | 0.04 | 0.20 | < 0.01 | 0.30 | 0.63 | 0.77 | 0.25 |
| Particulate Passage rate, %/h | 1.92 | 2.03 | 1.97 | 1.87 | 2.02 | 2.18 | 1.99 | 0.126 | 0.50 | 0.31 | 0.44 | 0.38 | 0.58 | 0.48 |
| pH | 6.7 | 6.7 | 6.6 | 6.4 | 6.9 | 6.6 | 6.6 | 0.09 | 0.33 | 0.07 | 0.01 | 0.66 | 0.90 | 0.22 |
| Ammonia, mMol/L | 1.4 | 2.2 | 7.6 | 11.2 | 2.4 | 3.1 | 7.4 | 0.78 | <0.01 | <0.01 | <0.01 | 0.52 | 0.02 | 0.07 |
| ***Day Before Supplementation*** | | | | | | | | | | | | | | |
| Particulate Fill, % BW | 0.97 | 1.09 | 1.01 | 1.02 | 0.95 | 0.91 | 0.90 | 0.06 | 0.85 | 0.02 | 0.34 | 0.55 | 0.88 | 0.75 |
| Particulate Passage rate, %/h | 1.54 | 1.92 | 2.01 | 1.77 | 1.86 | 1.91 | 1.97 | 0.144 | 0.03 | 0.90 | 0.92 | 0.52 | 0.38 | 0.52 |
| pH | 6.7 | 6.7 | 6.9 | 6.8 | 6.8 | 6.8 | 6.9 | 0.06 | 0.25 | 0.54 | 0.23 | 0.51 | 0.79 | 0.25 |
| Ammonia, mMol/L | 1.1 | 2.0 | 1.2 | 0.8 | 1.5 | 1.2 | 1.1 | 0.24 | 0.44 | 0.64 | <0.01 | 0.38 | 0.10 | 0.81 |

[a]

CON = control; D = 0.133 % body weight/day of soybean meal (SBM); 5D = 0.665% of body weight of SBM once every 5 days; 10D = 1.33% of body weight of SBM once every 10 days; 50% D = 50% of the D treatment; 50% 5D = 50% of the 5D treatment; 50% 10D = 50% of the 10D treatment.

[b] n = 4.

[c] Con vs Supp = control vs supplemented treatments; Full vs Half = full vs half amount of CP; L Freq = linear effect of supplementation frequency; Q SF = quadratic effect of supplementation frequency; L Freq vs Amt = interaction of the linear effect of supplementation frequency and amount of CP; Q Freq vs Amt = interaction of the quadratic effect of supplementation frequency and amount of CP.

**Table 4.** Effect of CP amount (soybean meal; SBM) and supplementation frequency on intake and diet digestibility by lambs.

| | Treatment[a] | | | | | | | | P-Value[c] | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Con | D | 5D | 10D | 50% D | 50% 5D | 50% 10D | SEM[b] | Con vs Supp | Full vs Half | L Freq | Q Freq | L Freq vs Amt | Q Freq vs Amt |
| Hay intake, % body weight | 1.88 | 2.16 | 1.95 | 1.40 | 2.02 | 1.93 | 1.87 | 0.173 | 0.97 | 0.45 | 0.02 | 0.60 | 0.08 | 0.52 |
| SBM intake, % body weight | 0.000 | 0.280 | 0.280 | 0.280 | 0.140 | 0.140 | 0.140 | | | | | | | |
| Total Intake, % body weight | 1.88 | 2.44 | 2.23 | 1.68 | 2.16 | 2.07 | 2.01 | 0.173 | 0.26 | 0.80 | 0.02 | 0.60 | 0.09 | 0.54 |
| Diet Digestibility, % | 37.4 | 40.6 | 45.7 | 49.6 | 43.5 | 43.5 | 43.5 | 1.98 | <0.01 | 0.28 | 0.04 | 0.86 | 0.04 | 0.87 |
| NDF Digestibility, % | 42.2 | 43.6 | 46.9 | 48.6 | 46.0 | 45.7 | 44.7 | 1.39 | 0.02 | 0.43 | 0.19 | 0.64 | 0.04 | 0.86 |
| CP Intake, % body weight | 0.092 | 0.240 | 0.230 | 0.205 | 0.165 | 0.165 | 0.160 | 0.0092 | <0.01 | <0.01 | 0.04 | 0.54 | 0.12 | 0.75 |
| CP Digestibility, % | 12.2 | 49.5 | 58.4 | 65.8 | 45.5 | 48.9 | 48.4 | 3.40 | <0.01 | <0.01 | 0.01 | 0.67 | 0.07 | 0.85 |
| Efficiency of CP Use, %[d] | -461 | 22 | 18 | 4 | 12 | 2 | 7 | 120 | <0.01 | 0.94 | 0.92 | 0.99 | 0.96 | 0.95 |
| Plasma Urea, mg/dL | 9.0 | 18.9 | 15.4 | 16.5 | 11.7 | 16.4 | 12.4 | 1.73 | <0.01 | 0.03 | 0.63 | 0.51 | 0.39 | 0.04 |

[a] CON = control; D = 0.280% of body weight/day of SBM; 5D = 1.4% of body weight of SBM once every 5 days; 10D = 2.8% of body weight of SBM once every 10 days; 50% D = 50% of the D treatment; 50% 5D = 50% of the 5D treatment; 50% 10D = 50% of the 10D treatment.
[b] n = 4.
[c] Con vs Supp = control vs supplemented treatments; Full vs Half = full vs half amount of CP; L Freq = linear effect of supplementation frequency; Q SF = quadratic effect of supplementation frequency; L Freq vs Amt = interaction of the linear effect of supplementation frequency and amount of CP; Q Freq vs Amt = interaction of the quadratic effect of supplementation frequency and amount of CP.
[d] Measured as the quantity of digested CP retained in the body.

**Table 5.** Effect of CP amount (soybean meal; SBM) and supplementation frequency on performance of cows in the last third of gestation.

| | Treatment[a] | | | | | | | | P-Value[c] | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Con | D | 5D | 10D | 50% D | 50% 5D | 50% 10D | SEM[b] | Con vs Supp | Full vs Half | L Freq | Q Freq | L Freq vs Amt | Q Freq vs Amt |
| ***Body Weight*** | | | | | | | | | | | | | | |
| Initial, lb | 1240 | 1241 | 1217 | 1231 | 1175 | 1260 | 1250 | 24.5 | 0.66 | 0.93 | 0.17 | 0.47 | 0.08 | 0.10 |
| Pre-Calving[d], lb | 1216 | 1311 | 1270 | 1227 | 1199 | 1265 | 1264 | 28.3 | 0.16 | 0.22 | 0.73 | 0.45 | < 0.01 | 0.47 |
| Post-Calving[e], lb | 1085 | 1179 | 1113 | 1115 | 1080 | 1123 | 1106 | 23.9 | 0.17 | 0.09 | 0.44 | 0.91 | 0.07 | 0.12 |
| Pre-Calving Change, lb | -23 | 70 | 53 | -4 | 24 | 6 | 15 | 12.7 | <.001 | 0.01 | < 0.01 | 0.76 | 0.01 | 0.11 |
| Post-Calving Change, lb | -155 | -62 | -104 | -116 | -95 | -137 | -143 | 15.1 | < 0.01 | < 0.01 | <.001 | 0.18 | 0.87 | 0.90 |
| ***BCS*** | | | | | | | | | | | | | | |
| Initial | 4.8 | 4.8 | 4.8 | 4.8 | 4.8 | 4.8 | 4.9 | 0.07 | 0.49 | 0.66 | 0.73 | 0.51 | 0.76 | 0.47 |
| Pre-Calving | 4.4 | 4.9 | 4.8 | 4.6 | 4.6 | 4.6 | 4.6 | 0.10 | 0.02 | 0.05 | 0.08 | 0.97 | 0.06 | 0.51 |
| Post-Calving BCS | 4.1 | 4.6 | 4.6 | 4.4 | 4.5 | 4.4 | 4.4 | 0.09 | <.001 | 0.34 | 0.14 | 0.61 | 0.21 | 0.51 |
| Pre-Calving BCS | -0.4 | 0.1 | 0.0 | -0.3 | -0.2 | -0.2 | -0.3 | 0.09 | 0.027 | < 0.01 | 0.02 | 0.53 | 0.05 | 0.90 |
| Post-Calving BCS | -0.6 | -0.2 | -0.3 | -0.5 | -0.4 | -0.4 | -0.5 | 0.11 | 0.009 | 0.26 | 0.13 | 0.38 | 0.40 | 0.96 |

[a] CON = control; D = 1.02 lb/head of SBM daily; 5D = 5.1 lb/head of SBM once every 5 days; 10D = 10.2 lb/head of SBM once every 10 days; 50% D = 50% of the D treatment; 50% 5D = 50% of the 5D treatment; 50% 10D = 50% of the 10D treatment.
[b] n = 4.
[c] Con vs Supp = control vs supplemented treatments; Full vs Half = full vs half amount of CP; L Freq = linear effect of supplementation frequency; Q SF = quadratic effect of supplementation frequency; L Freq vs Amt = interaction of the linear effect of supplementation frequency and amount of CP; Q Freq vs Amt = interaction of the quadratic effect of supplementation frequency and amount of CP.
[d] Measured within 14 days prior to calving.
[e] Measured within 24 hours after calving.

Oregon State University

BEEF088

Beef Cattle Sciences

Beef Research Report

# Sampling Large Lots of Hay for Nutritional Analyses – Subsampling to Reduce Collected Sample Size is Acceptable[1]

D. W. Bohnert[2], R. F. Cooke[2], B. I. Cappellozza[2], C. Trevisanuto[2], and V. D. Tabacow[2]

## Synopsis

Subsampling cored hay samples that are greater than 0.5 pound by spreading the sample over 9 quadrants, arranged in a tic-tac-toe layout, and collecting 33% of the original sample volume (3 quadrants) does not bias nutritional results and is an effective way to reduce sample size for laboratory analysis.

## Summary

When sampling large lots of hay for nutrient analyses, the number and quantity of cores required to obtain a representative sample often results in producers arbitrarily subsampling in order to reduce the volume of sample sent to a testing lab. This can bias results due to improper subsampling technique; consequently, we compared 2 methods of sampling 4 different baled hays from eastern Oregon. We obtained 2 cores (A & B) from each bale, 5 inches apart, from 4 lots of 20 bales of each forage type. The A & B cores were grouped by forage type within lot. The first method used 100% of the A cores from each lot (CON) and the second method involved subsampling the B cores from each lot via a quadrant method (SUB) in which the cores were mixed well, spread out on a plywood sheet labeled with 9 quadrants (5 × 5 in), and approximately 33% of the overall sample was obtained for analyses. No differences were noted between CON and SUB or the interaction of sampling method and forage type for NDF, ADF, TDN, and CP; differences were noted due to forage type for each nutrient. The take home message from this data is that the CON and SUB values for NDF (61.4 vs 61.2%), ADF (32.1 vs 31.9%), TDN (58.2 vs 58.4%), and CP (12.0 vs 12.1%) were not affected by sampling procedure. We do not recommend routine subsampling of cored hay samples; however, these data indicate that subsampling can be used to reduce sample size if proper attention to procedures is followed.

## Introduction

Hay sampling and nutritional analyses are important components of most nutritional programs for ruminant livestock. This information is critical for ration formulation, determining hay value, and allocating hays within an operation's inventory to the appropriate classes of livestock.

A common question when sampling hay is how many bales must be sampled to get a representative sample of the lot of hay. The National Forage Testing Association (NFTA; Putnam, 2011; Putnam and Orloff, 2011) recommends a minimum of 20 bales (one core sample per bale) with up to 35 bales for large lots (100 to 200 ton) or if hay nutritional quality is expected to be very variable. In addition, NFTA strongly recommends that core samples for each lot of hay are combined into a single sample, not subsampled, and sent to a laboratory for testing.

1. This document is part of the Oregon State University – 2012 Beef Research Report. Please visit the Beef Cattle Sciences website at http://beefcattle.ans.oregonstate.edu.
2. Oregon State University - EOARC, Burns 97720.

Depending on the coring device, this can result in a large volume of sample collected. Nevertheless, NFTA also suggests that the sample of cores from each lot of hay weigh approximately 0.5 lb (Putnam, 2011; Putnam and Orloff, 2011) which may not be possible when using some probes and/or with large lots of hay. Furthermore, most forage testing laboratories request that from 8 to 20 bales be sampled for each lot of hay and/or suggest that each group of cores from a lot of hay fit within a "gallon" bag. This is to minimize the volume of sample the laboratories must process prior to analysis. Consequently, with large lots of hay or hay that is assumed to be highly variable in nutrient content, individuals or laboratories often manually subsample when the number of cores collected yields greater than 0.5 lb. This can result in improper subsampling and nutrient analyses that are not representative of the lot of hay.

Consequently, we designed a study to evaluate a subsampling procedure for cored hay samples. If successful, this procedure will allow for reduction of sample size while not affecting nutrient analyses compared with hay cores that are not subsampled.

## Materials and Methods

We obtained core samples using a Penn State Sampler (Nasco, Fort Atkinson, WI) from 4 baled hays common to eastern Oregon. The hays were alfalfa (3 × 4 × 8 ft bales), grass/alfalfa (2-tie small bales), Chewings fescue grass seed straw (3 × 4 × 8 ft bales), and meadow foxtail (5 ft diameter round bales). We obtained 2 cores (A & B) from each bale, 5 inches apart, from 4 lots of 20 bales of each hay type. Coring technique followed the procedure recommended by NFTA (Putnam, 2011). The A & B cores were grouped by hay type within lot.

The first sampling method used 100% of the A cores from each lot (CON) and the second method involved subsampling the B cores from each lot via a quadrant method (SUB) in which the cores were mixed well, piled in the middle of a plywood sheet labeled with 9 quadrants (5 × 5 inches) and spread to cover all quadrants, and approximately 33% of the overall sample (the middle, vertical column of a tic-tac-toe arrangement) was obtained for analyses. Samples were dried (130°F; 96 h), ground, and analyzed for CP (Leco CN-2000; Leco Corp., St. Joseph, MI) and NDF (Robertson and Van Soest, 1981) and ADF (Goering and Van Soest, 1970) using procedures modified for use in an Ankom 200 Fiber Analyzer (Ankom Co., Fairport, NY). In addition, TDN was estimated for all forages [82.38-(0.7515*ADF)].

Data were analyzed with the MIXED procedure of SAS (SAS Inst., Inc., Cary NC). The model included sampling method, hay type, and the resultant interaction with degrees of freedom calculated by the Satterthwaite procedure. In addition, replication within hay type was used to specify variation using the RANDOM statement. The LSMEANS were separated using LSD protected by a significant F-test ($P \leq 0.05$).

## Results

Differences in hay type were observed for CP, NDF, ADF, and TDN ($P < 0.001$; data not shown); however, no differences were noted for the interaction of method × hay type (Table 1; $P \geq 0.09$) or sampling method ($P > 0.30$). Consequently, overall CON and SUB LSMEANS for CP, NDF, ADF, and TDN were, on a DM basis, 12.0 vs 12.1% (SEM = 0.22), 61.4 vs. 61.2% (SEM = 0.28), 32.1 vs. 31.9% (SEM = 0.31), and 58.2 vs. 58.4% (SEM = 0.23), respectively. These data indicate subsampling using the procedure described herein is an acceptable method to reduce sample size without biasing results compared with cores that were not subsampled.

Nutrient analyses can only be as good as the sample collected. Therefore, it is critical to obtain a representative sample from each lot of hay. Unfortunately, there is no definitive recommendation for the number of bales to sample for nutrient analysis with respect to varying lot size and hay type. A study from Kansas State University provides sampling recommendations for 99%, 95%, and 80% confidence intervals for the CP content of alfalfa, prairie hay, and sorghum-sudan hay determined to within 1% or 0.5% CP of the actual mean (Blasi, 2011). The recommendations are specific to each forage type; however, the general recommendation is to sample 20% of the bales in a lot of hay to obtain a representative sample for CP analysis. However, the most commonly accepted recommendation by the forage industry is to use a minimum of 20 bales (one core per bale) and to sample more bales for larger lots of hay or if the hay is assumed to be very variable in nutrient composition (NFTA; Putnam, 2011; Putnam and Orloff, 2011).

The National Forage Testing Association recommends that the amount of sample obtained from each lot of hay be approximately 0.5 lb to assure that the amount of sample is an easily managed and processed size (Putnam, 2011; Putnam and Orloff, 2011). This may not be possible for large lots of hay or hay that is highly variable in nutrient composition. Consequently, many hay growers, livestock owners, nutritionists, and forage testing laboratories subsample when samples from a lot of hay exceed 0.5 lb. Even though this is not a recommended practice by NFTA (Putnam, 2011; Putnam and Orloff, 2011), our data suggests that the subsampling method described herein can be an acceptable practice with cored hay samples greater than 0.5 lb.

## Conclusions

In conclusion, this work demonstrates that using the procedure described herein can be an effective process to subsample large lots of hay while not biasing nutritional results compared with non-subsampled data. Consequently, this allows producers to obtain more cores from large lots of hay, subsample, and still maintain the quantity of sample to be analyzed within the industry recommended guideline of 0.5 lb.

## Literature Cited

Blasi. 1998. Forage Facts Notebook. Kansas State Univ. Res. & Ext.

Goering and Van Soest. 1970. Agric. Handbook No. 379. ARS, USDA, Washington, D.C.

Putnam. 2012. Forage Sampling. National Forage Testing Association.

Putnam and Orloff. 1998. California Alfalfa and Forage Review.

Robertson and Van Soest. 1981. The Analysis of Dietary Fiber. Pages 123-158.

**Table 1.** Influence of sampling method and hay type on nutrient concentration (DM basis). [a]

| | Hay | | | | | |
|---|---|---|---|---|---|---|
| Nutrient, % | Alfalfa | Alfalfa/Grass | Grass | Grass Seed Straw | SEM[b] | *P*-Value[c] |
| CP | | | | | 0.22 | 0.70 |
| Control | 21.8 | 15.4 | 5.2 | 5.6 | | |
| Subsample | 21.2 | 15.6 | 5.2 | 6.4 | | |
| NDF | | | | | 0.28 | 0.31 |
| Control | 43.9 | 58.9 | 64.7 | 78.1 | | |
| Subsample | 43.5 | 57.7 | 65.0 | 78.4 | | |
| ADF | | | | | 0.31 | 0.42 |
| Control | 25.8 | 27.9 | 32.7 | 42.1 | | |
| Subsample | 25.8 | 27.1 | 32.8 | 42.1 | | |
| TDN | | | | | 0.23 | 0.42 |
| Control | 63.0 | 61.4 | 57.8 | 50.8 | | |
| Subsample | 63.0 | 62.0 | 57.7 | 50.8 | | |

[a] 2 cores (A & B) were obtained from each bale, 5 inches apart, from 4 lots of 20 bales of each hay type. The A & B cores were grouped by hay type within lot. The first method used 100% of the A cores from each lot (Control) and the second method involved subsampling the B cores from each lot in order to obtain approximately 33% of the original sample volume (Subsample).

[b] n = 4; Method Effect SEM

[c] Method Effect; no Method × Hay Interaction ($P \geq 0.09$).

Oregon State University | Extension Service

**Oregon State University**

**Beef Cattle Sciences**


**We acknowledge Flavia Cooke for replicating and assembling this report.**