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Analysis of a Sheep Cover Crop Grazing Trial in Southwestern North Dakota[1]

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The objective of this study was to evaluate forage production and potential for late-season sheep gains on a single-cropped cover crop planting in Southwestern North Dakota in hopes to increase forage availability later into the fall and subsequently reduce feedlot dependency. Our research suggests there was little difference in ewe body weight gain for ewes grazing two cover crop mixtures, but ewes grazing mixed-grass prairie exhibited a significant reduction in ADG when compared to the ewes grazing cover crops.

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SUMMARY

Determining forage value and potential livestock production from cover crop plantings gives sheep producers an alternative to supplemental feeding of ewes grazing rangelands or being forced to enter into drylot feeding, hopefully decreasing both labor and feed costs. Cover crops provide numerous environmental benefits, including soil health improvements, increased soil moisture for future crop yields, and as an excellent food and cover source for many wildlife species. Although cover crops have related expenses, the environmental benefits, coupled with the availability of late-season forage, may make them appealing to farm/ ranch operations.

During this study, bred brood ewes were placed in one of nine different paddocks with a total of three different treatments during October (2010, 2011, and 2012). Treatments consisted of two different spring cover crop plantings and an idled mixed-grass prairie paddock that served as a control. Ewes gained an average of 0.28 lbs/day on the cover crop plantings and lost approximately 0.03 lbs/day on the mixed-grass control. Our research suggests that standing cover crops can provide substantial forage with adequate nutritional value to bred ewes to length the grazing season, delaying the onset of supplemental feeding or entry into the drylot.

INTRODUCTION

Finding ways to increase the length of the grazing system is a common way for livestock managers to reduce feed costs (Adams et al., 1994). Grazing annual forages as a supplemental late-season food source for livestock can serve as a way to increase the grazing season while providing high-quality forages for livestock (Neville et al., 2008). Cover crops have grown in popularity across much of the US as a way to provide multiple benefits to farm lands, including nutrient cycling efficiency and soil and water conservation (Franzluebbers and Stuedemann, 2006). Livestock grazing of cover crops has had variable effects on soil quality and subsequent crop production, but overall has shown increased economic returns and diversity (Franzluebbers and Stuedemann, 2006; Bell et al., 2011). Our study assessed the forage suitability of two different cover crop mixes compared with mixed-grass prairie for gestating Rambouillet ewes.

PROCEDURES

All procedures were approved by the NDSU Animal Care and Use Committee. The study was conducted at the Hettinger Research Extension Center near Hettinger, North Dakota, in Adams County. The study area receives approximately 16 inches of precipitation annually, with the average summer temperature (June through August) approximately 66°F (NDAWN, 2012).

Grazing Treatments. The grazing study utilized three different treatments randomly allotted to 9 paddocks (1.6 ac; n = 3): cover crop treatment 1, designed as a species mixture targeting pollinators (insects), wildlife, and soil health benefits (**CC1**), cover crop treatment 2, designed as a forage crop for livestock and for soil health benefits (**CC2)**, and the mixed-grass range control consisting of smooth bromegrass, crested wheatgrass, and alfalfa (**CON**). The CC1 treatment utilized seed mixtures containing 16, 9, 2, 2, 1.6, 1, and 0.6 lb/ac for oats, forage soybean, Proso millet, milo, purple-top turnip, sweet clover, and forage radish, respectively ($26.36/ac.). The CC2 treatment utilized planting rates of 3.6, 3, and 1.6 lb/ac for purple-top turnips, Proso millet, and forage radish, respectively ($22.22/ac). CC1 and CC2 treatments were annually sprayed with glyphosate prior to planting. Planting occurred in mid-June, with fertilizer (11-52-0) applied to both CC1 and CC2 at 50 lb/ac at the time of planting.

Vegetative data was collected at the onset of the research trial during each of the three years. Peak production was determined in late -July in each paddock by species for each species present using nine 1/4 m^2 frames per paddock and extrapolated to determine average total lbs/ac/species for each treatment. Concurrently, vegetation clippings were dried and sent to Midwest Laboratories Inc. for nutrient analysis.

Animals. One hundred and eight Rambouillet ewes bred to lamb approximately on January 15 were utilized to evaluate livestock performance. Two-day weights were taken at the beginning and end of the grazing period. Ewes were stratified by weight and randomly assigned to one of 9 paddocks (12 ewes per paddock). Each of the nine paddocks was grazed for approximately 30 days in October during 2010, 2011, and 2012.

Statistical Analysis. Data were analyzed using the MIXED procedure of SAS (SAS Inst., Inc, NC). Paddock served as the experimental unit (n = 3). The covariance structure was Autoregressive. The fixed effect included in the model was treatment. Treatment, year, and treatment x year interactions were evaluated. When a significant F-test was observed ($P \leq 0.05$), LS Means was used to partition effects. Significance was determined at $P \leq 0.05$. All interactions that were not significant were removed from the model.

RESULTS AND DISCUSSION

Animals and forage production. Treatment, year, and treatment x year effects are listed in Table 1. Average daily gain of pregnant Rambouillet ewes was significantly affected ($P = 0.02$) by treatment, with a treatment by year interaction ($P = 0.91$) present, and a year effect ($P < 0.01$). Average daily gain was higher for CC1 and CC2 than CON (0.27, 0.30, and -0.03 lb/d, respectively). Forage quantity did not appear to be the reason for this difference, as no effects were observed for treatment, year, or treatment x year ($P \geq 0.76$).

Nutrient Analysis. Nutrient analysis of CC1, CC2, and CON are listed in Table 1. Treatment x year interactions were observed ($P \leq 0.02$) for CP, TDN, NE_m, and NE_g. While variable across years, CC1 and CC2 tended to have higher CP concentrations relative to CON (11.84, 12.04, and 5.9%, respectively). This largely explains differences in body weight gains across treatments, as energy (expressed as TDN, NE_m, and NE_g) was not affected by treatment ($P \geq 0.19$), even though it was variable across the treatment x year interaction ($P \leq 0.01$). Additionally, ADF was greater ($P < 0.01$) for CON (44.97%) compared to CC1 and CC2 (30.94 and 27.99%, respectively), further explaining differences in performance.

Mineral Analysis. Treatment x year interactions were observed for Ca and Cu ($P \leq 0.02$). Similar to nutrient concentrations, variability existed between years, especially for the cover crop treatments (Table 1). However, CON had consistently lower Ca and Cu concentrations than CC1 and CC2. This trend for increasing concentrations of minerals in cover crop treatments is also present

for S, P, K, Mg, and Zn, which exhibited a treatment effect ($P \leq 0.03$). However, two minerals, Fe and Mn exhibited a treatment effect ($P \leq 0.03$) in which CON was similar ($P \geq 0.05$) to either CC1 or CC2. In general, the cover crop treatments resulted in mineral concentrations that would be expected for grain-type annual forages, of which many were present in the cover crop plant seeding mixtures.

IMPLICATIONS

Cover crop plantings, either targeting wildlife use or forage for livestock, resulted in ADG in pregnant ewes that were significantly higher than when ewes grazed mixed-grass prairie in the early fall. These results suggest further research should be conducted to determine optimal planting mixtures and timing of grazing of cover crops being utilized as soil health amendments, wildlife habitat, and forage for sheep grazing.

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Table 1. Sheep production, vegetative biomass production, and feed nutrition analysis from a sheep cover crop grazing trial in Adams County, North Dakota, October 2010, 2011, and 2012[1]

	Cover Crop 1[2]				Cover Crop 2[3]				Mixed-Grass Prairie[4]					*P*-value		
Items	2010	2011	2012	Avg	2010	2011	2012	Avg	2010	2011	2012	Avg	SEM[Trt]	Trt	Yr	Trt*Yr
ADG, #/d	0.66	-0.04	0.18	0.27^{x}	0.70	-0.09	0.30	0.30^{x}	0.42	-0.49	-0.03	-0.03^{y}	0.08	0.02	<0.01	0.91
Biomass, #/ac.	1966	2317	2356	2213	2200	1900	2252	2117	1982	1845	2015	1947	255	0.76	0.84	0.96
Nutrient Analysis																
CP, %	14.72^{de}	10.33^{bc}	10.49^{bcd}	11.84	15.17^{e}	7.97^{ab}	13.00^{cde}	12.04	6.22^{ab}	5.59^{a}	5.89^{a}	5.90	0.84	<0.01	0.13	0.02
Crude Fat, %	1.88	2.28	2.50	2.22	1.54	1.80	1.77	1.70	1.91	2.45	1.56	1.98	0.21	0.09	0.61	0.43
ADF, %	24.2	33.4	35.23	30.94^{y}	23.00	29.2	31.77	27.99^{y}	43.17	44.33	47.4	44.97^{x}	1.82	<0.01	0.13	0.65
Ash, %	11.06	8.04	6.08	8.93^{x}	12.13	7.07	8.79	9.33^{x}	8.44	6.19	6.32	6.98^{y}	0.47	<0.01	<0.01	0.11
TDN, %	63.07^{ab}	65.07^{cd}	66.33^{de}	64.82	62.23^{a}	66.83^{e}	64.03^{abc}	64.37	64.07^{bc}	65.90^{cde}	65.07^{cde}	65.01	0.37	0.21	0.02	<0.01
NE_M, Mcal/lb	0.64^{ab}	0.67^{cd}	0.69^{de}	0.66	0.63^{a}	0.69^{e}	0.66^{abc}	0.66	0.66^{bc}	0.68d^{e}	0.67cd^{e}	0.67	0.01	0.19	0.03	<0.01
NE_G, Mcal/lb	0.37^{ab}	0.40^{cd}	0.42^{de}	0.39	0.35^{a}	0.42^{e}	0.38^{abc}	0.39	0.38^{bc}	0.41^{ed}	0.40^{cde}	0.40	0.01	0.21	0.01	<0.01
Mineral Analysis																
S, %	0.343	0.313	0.143	0.27^{xy}	0.483	0.247	0.447	0.39^{x}	0.113	0.103	0.090	0.10^{y}	0.064	0.02	0.58	0.50
P, %	0.310	0.247	0.217	0.26^{x}	0.357	0.340	0.260	0.32^{y}	0.150	0.130	0.080	0.12^{z}	0.016	<0.01	0.01	0.86
K, %	2.71	1.98	1.55	2.08^{x}	2.70	1.77	1.65	2.04^{x}	0.52	0.54	0.22	0.43^{y}	0.11	<0.01	<0.01	0.15
Mg, %	0.420	0.350	0.333	0.37^{x}	0.523	0.313	0.393	0.41^{x}	0.117	0.157	0.093	0.12^{y}	0.040	<0.01	0.51	0.20
Ca, %	2.26^{b}	0.76^{a}	0.54^{a}	1.19	2.87^{b}	0.58^{a}	0.97^{a}	1.48	0.47^{a}	0.61^{a}	0.39^{a}	0.49	0.15	<0.01	<0.01	<0.01
Na, %	0.025	0.043	0.005	0.02	0.085	0.028	0.080	0.06	0.005	0.005	0.005	0.01	0.021	0.17	0.90	0.77
Fe, ppm	101.3	85.67	196.7	127.9^{y}	195.0	140.3	375.3	236.9^{x}	135.7	107.0	249.3	164.0^{y}	22.33	0.01	0.01	0.54
Mn, ppm	47.7	51.7	50.0	49.78^{y}	104.3	52.0	90.7	82.33^{x}	87.3	63.0	91.7	80.0^{x}	9.84	0.03	0.38	0.31
Cu, ppm	3.00^{abc}	3.67^{bcd}	6.00^{f}	4.22	4.33^{cde}	4.67^{def}	5.67^{ef}	4.89	2.67^{ab}	3.00^{abc}	2.00^{a}	2.56	0.28	<0.01	0.03	0.02
Zn, ppm	28.0	24.0	27.3	26.44^{y}	37.33	43.00	34.33	38.22^{x}	23.33	20.00	26.67	23.33^{y}	3.10	0.03	0.99	0.77

[1]Bolded items indicate main and interaction effects with highest order of significance.
[2]Cover crop mixture for pollinators (insects), wildlife, and soil health benefits (**CC1**).
[3]Cover crop mixture for livestock forage and soil health benefits (**CC2).**
[4]Mixed-grass prairie consisting of smooth bromegrass, crested wheatgrass, and alfalfa (**CON**).
[a,b,c,d,e] Means within a row, with a significant Trt*Yr interaction, without a common superscript differ ($P \leq 0.05$).
[x,y,z] Means within a row, with a significant Trt effect, without a common superscript differ ($P \leq 0.05$).

Influence of the level of dried distillers grains with solubles on feedlot performance, carcass characteristics, blood metabolites, and semen quality of growing rams[1]

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The objectives of this research were to determine the influence of dried DDGS on feedlot performance, carcass characteristics, semen quality, and testosterone concentrations of growing rams. Results indicate that semen quality may be affected by increasing concentrations of DDGS in rations fed to growing rams.

INTRODUCTION

In the past 10 years, ethanol production has increased from 1.5 million gallons per year to approximately 9 million gallons per year in the United States (Renewable Fuels Association, 2010). With this expansion brings an affordable and viable feed source for ruminants, dried distillers grains with solubles (DDGS). Anecdotal reports from the feed industry have reported that growing bulls and rams should not be fed DDGS, due to a fear of a negative effect on reproductive performance. To our knowledge, there is no research currently available describing the effect of DDGS feeding on male reproductive performance.

Research involving the feeding of DDGS to ruminants has become more prominent in the past few years due to the rising costs of feedstuffs, particularly corn. Compared with non-supplemented heifers, dried distiller's grains supplemented heifers had increased ADG and reduced forage intake (MacDonald et al., 2007). However, during the growing and finishing phase DDGS fed to steers at 30% of the diet did not affect any performance variable or carcass characteristic (Leupp et al., 2009). Similarly, Schauer et al. (2008) and Neville et al. (2010) observed no negative effects on finishing lamb performance or carcass characteristics when fed DDGS at 60%.

While we are not aware of research evaluating the effects of DDGS feeding on male reproductive performance, there is some research available on feeding increased dietary CP to growing males (an artifact of increasing DDGS in rations, as DDGS is relatively high in CP). Rams fed a high energy and protein diet had increased testosterone concentrations at the beginning of the trial, but as the trial duration increased, the differences in testosterone concentrations were reduced (Martin et al., 1994). Hotzel et al. (1998) observed an increase in testosterone concentrations in Merino rams fed a diet above maintenance requirements.

Therefore, we hypothesized that feeding increasing levels of DDGS would have no deleterious effects on ram feedlot performance, carcass characteristics, and semen quality, but would increase testosterone concentrations.

[1]This project was supported by North Dakota Corn Council. The authors would like to thank David Pearson, Krista Cella, Karla Ryan, Nicole Engraf, Sulley Merrwin, Donald Drolc, and Don Stecher for their assistance in conducting this trial.

PROCEDURES

All procedures were approved by the Animal Care and Use Committee of North Dakota State University. This study was conducted at the Hettinger Research Extension Center in Hettinger, North Dakota.

Animals and Diets. One hundred twenty crossbred rams (western whiteface x Suffolk; approximately 90 d of age) were used in a completely randomized design to determine the effects of DDGS on feedlot performance, carcass characteristics, semen quality, and testosterone concentrations of growing rams. Rams were allotted into one of four dietary treatments (n = 4 pens/treatment; 10 rams/pen; Table 1): 1) **CON**: 85% corn and 15% commercial market lamb pellet; 2) **15DDGS**: 15% DDGS substituted for corn (DM basis); and 3) **30DDGS**: 30% DDGS substituted for corn (DM basis). Rams were fed a ground ration (grinder-mixer) ad-libitum via self-feeders. Rams had continuous access to water and shade. Rams were weighed on two consecutive days at the beginning (d 0, 1) and end of the trial (d 96, 97 and d 116, 117), and weighed on a single day every 28 d. Scrotal circumference was measured on d 84, 96, and 116 of the trial. Two slaughter dates were utilized for the trial. The first slaughter date included all rams weighing at least 67 kg except those involved with the semen quality and testosterone portions of the trial. The second slaughter date included all remaining rams on trial. At completion, rams were shipped to Superior Farms in Denver, CO for carcass data collection. One ram was removed from the trial prior to being shipped for slaughter due to non-treatment related purposes (antibiotic withdrawal time).

Semen Quality. Semen was collected on forty-eight rams (a subsample of the 120 rams in the feedlot study described above; 4 rams/pen; 16 rams/treatment; n = 4). Semen from each of the rams was collected on d 84, 98, and 112 of the study. Motility, a subjective motility score, and concentration of sperm in the ejaculate via a hemocytometer on the fresh ejaculate sample were used to determine semen quality.

Testosterone. The forty-eight rams (4 rams/pen; 16 rams/ treatment; n = 4) utilized in semen collection were used to collect testosterone concentrations. Blood samples were collected via a 20 gauge x 1 inch vacutainer needles into serum separator 16 x 100 mm tubes. Every 14 days throughout the duration of the trial, a 10 mL blood sample was collected via jugular venipuncture of each ram and immediately placed on ice until serum could be harvested post-centrifugation. Serum was frozen at -20ºC until analysis could be accomplished.

Statistical Analysis. Ram feedlot performance, carcass characteristics, and scrotal circumference were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). Pen served as the experimental unit. The fixed effect included in the model was dietary treatment. The fixed effect of day was utilized in the REPEATED measures analysis for testosterone concentrations, spermatozoa concentration, and the subjective semen score. The model included the fixed effects of dietary treatment, week, and treatment x week. When a significant F-test was observed ($P \leq 0.15$), preplanned comparisons of linear and quadratic contrasts were utilized to partition treatment effects. Significance was determined at $P \leq 0.05$. All interactions that were not clearly significant ($P \geq 0.20$) were removed from the model. To partition day effects and treatment × day interactions, LS Means was utilized ($P \leq 0.05$).

RESULTS AND DISCUSSION

Feedlot and Carcass Characteristics. Final BW and days on study were not affected ($P \geq 0.50$) by dietary treatment (Table 2). Average daily gain increased linearly ($P = 0.02$) with the addition of DDGS in the diet. Previous research has also suggested that lambs consuming rations containing DDGS have increased ADG compared with those lambs consuming no DDGS (Schauer et al., 2008). Overall, DMI increased linearly ($P < 0.001$) as the amount of DDGS was increased in the ration. These results are similar to those observed by Schauer et al. (2008) when DDGS inclusion was increased to 60% of the ration as a replacement for barley. However, G:F was reduced linearly ($P < 0.001$) with the inclusion of DDGS in the diet. Although there were some significant differences, the DDGS did not cause any overall deleterious effects on feedlot performance. This is indicated by the lack of differences in the amount of days on trial among the dietary treatments.

Hot carcass weight, dressing percentage, ribeye area, 12th rib fat

thickness, body wall thickness, leg score, overall conformation, flank streaking, quality grade, yield grade, and percent boneless, closely trimmed retail cuts were not affected ($P = 0.26$; Table 2) by dietary treatment. These results were similar to Schauer et al. (2008) and Neville et al. (2010) in which there were no deleterious effects on carcass characteristics with DDGS inclusion in the diet.

Reproductive Traits. Change in scrotal circumference was not significant ($P = 0.61$) due to dietary treatment (Table 2). Contrary to the results in the current study, Martin et al. (1994) noted an increase in scrotal circumference in the high protein and energy fed rams compared with the low energy and protein fed rams. Hötzel et al. (1998) observed an increase in the change in scrotal circumference throughout the study in the rams fed to have increased rate of gain. Similar results were also observed in bulls, in which bulls fed increased energy diets (Coulter and Kozub, 1984). Although TDN was not different between diets, the CP of the diets increased with the increasing DDGS. Therefore, the results in the current study were not expected. There was a day effect ($P < 0.001$; Figure 1) for testosterone concentrations. This was expected as rams became more mature throughout the study; therefore, the testosterone concentrations would be expected to increase as the rams reached maturity. Testosterone concentrations were decreased in mature Merino rams fed a sub-maintenance diet compared with those fed a supra-maintenance (Hötzel et al., 1998). Martin et al. (1994) observed similar results to Hötzel et al. (1998), in which the high and intermediate energy and protein fed rams had increased testosterone concentrations compared with the low energy and protein fed rams.

Spermatozoa concentration decreased linearly ($P = 0.05$; Table 2) as DDGS increased in the diet. The rams fed the DDGS at both 15 and 30% had numerically reduced spermatozoa numbers compared with the rams that were not fed any DDGS. Coulter and Kozub (1984) observed a reduction in epididymal spermatozoa reserves and motility in bulls fed a high energy diet. The current results as well as previous research (Kozub and Coulter, 1984), may suggest increased protein and/or fat cause a reduction in spermatogenesis. This may be due to increased fat deposits around the seminiferous tubules. The spermatozoa motility score not affected ($P = 0.23$) by DDGS level (Table 2).

IMPLICATIONS

Much of the previous research with male reproductive performance has occurred in mature rams and bulls. Therefore, much of the data does not include growing rams as they approach and reach puberty. The current research suggests that feedlot rams can be fed up to 30% on DM basis of DDGS without causing deleterious effects to feedlot performance and carcass characteristics. However, care must be taken when feeding DDGS to growing rams due to a possible reduction in spermatozoa concentration, especially when included at 15% of the diet or higher. Further research is needed to elucidate why semen quality may be affected and if actual fertility of rams is compromised by feeding increasing concentrations of DDGS.

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Table 1. Ingredient and nutritional composition of diets fed to feedlot ram lambs (DM basis).

	Dietary Treatment[1]		
Item	CON	15DDGS	30DDGS
Ingredient, %			
Corn	85	70	55
DDGS[2]	—	15	30
Commercial market lamb pellet[3]	14.8	14.3	13.8
Calcium carbonate	0.2	0.7	1.2
Nutritional Composition, % DM			
TDN	84.6	84.6	84.3
CP	13.8	16.0	19.4
Ash	4.7	5.5	6.4
NDF	18	22.2	26.1
ADF	4.6	5.5	5.7
Crude Fat	2.3	3.7	4.6

[1]CON: 85% corn and 15% commercial market lamb pellet; 15DDGS: 15% DDGS substituted for corn on a % DM basis; and 30DDGS: 30% DDGS substituted for corn on a % DM basis.
[2]Dried distiller's grains with solubles.
[3]Commercial Market Lamb Pellet contained: 0.22 g/kg Chlortetracycline; 38.0% CP; 3.75-4.75% Ca; 0.6% P; 3.0-4.0% salt; 1.2 ppm Se; 52,863 IU/lb Vitamin A; 5,286 IU/kg Vitamin D; and 209 IU/kg Vitamin E.

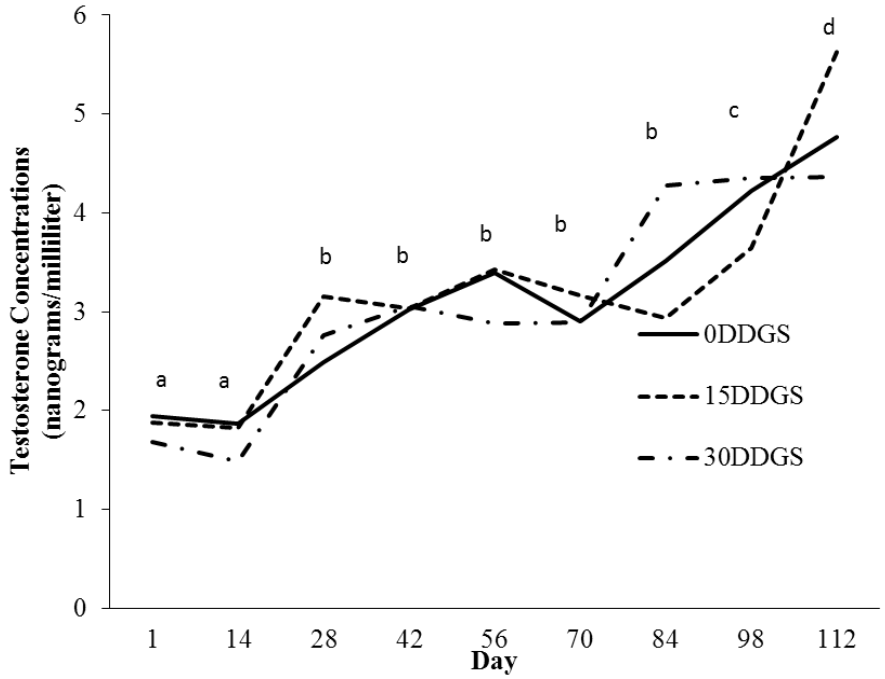

Figure 1. The effects of dried distillers grains with solubles on testosterone concentrations of growing ram lambs. Treatments included CON: 85% corn and 15% commercial market lamb pellet; 15DDGS: 15% DDGS substituted for corn (DM basis); and 30DDGS: 30% DDGS substituted for corn (DM basis). *P*-values: treatment, $P = 0.97$; day, $P < 0.001$; treatment x day, $P = 0.86$.

Table 2. Effects of dried distiller's grains with solubles on feedlot performance and carcass characteristics of growing rams.

	Dietary Treatment[1]					*P*-value[4]	
Item	CON	15DDGS	30DDGS	SEM[2]	*P*-value[3]	Linear	Quadratic
Initial BW, lb	91.03	89.56	89.03	3.11	0.89	0.65	0.90
Final BW, lb	184.40	185.17	190.33	3.91	0.50	0.28	0.64
ADG, lb/d	0.96	0.98	1.04	0.02	0.06	0.02	0.52
DMI, lb/head/d	4.53	5.16	5.58	0.14	0.001	< 0.001	0.55
Days on study, d	109	108	107	1.59	0.54	0.27	0.90
G:F, lb of gain/lb of DMI	0.43	0.38	0.38	0.01	< 0.001	< 0.001	0.09
HCW, lb	91.91	93.74	93.82	2.46	0.81	0.57	0.77
Dressing %	49.95	50.41	50.13	0.37	0.67	0.72	0.41
REA, in^2	3.05	3.09	3.08	0.07	0.90	0.74	0.74
12th rib fat thickness, in	0.22	0.22	0.21	0.01	0.88	0.69	0.76
Bodywall thickness, in	1.05	1.11	1.13	0.04	0.26	0.11	0.70
Leg score[5]	11.60	11.98	11.69	0.25	0.54	0.80	0.29
Overall conformation[5]	11.60	11.96	11.80	0.21	0.47	0.49	0.32
Flank streaking[6]	350.79	374.88	356.86	11.57	0.30	0.70	0.14
Quality grade[5]	11.56	11.89	11.82	0.16	0.29	0.24	0.30
Yield grade	2.55	2.56	2.48	0.14	0.88	0.69	0.76
BCTRC,[7] %	44.98	44.73	44.87	0.30	0.84	0.79	0.60
Scrotal circumference change, in	0.59	0.49	0.68	0.74	0.61	0.65	0.39
Spermatozoa concentration[8]	91.8	69.3	63.0	10.17	0.13	0.05	0.52
Spermatozoa motility score[9]	3.3	2.8	2.7	0.23	0.23	0.12	0.52

[1]CON: 85% corn and 15% commercial market lamb pellet; 15DDGS: 15% DDGS substituted for corn (DM basis); and 30DDGS: 30% DDGS substituted for corn (DM basis).
[2]n = 4.
[3]*P* -value for the F test of the mean.
[4]*P*-value for linear and quadratic effects of increasing dried distillers grains with solubles.
[5]Leg score, conformation score, and quality grade: 1 = cull to 15 = High Prime.
[6]Flank streaking: 100-199 = practically devoid; 200-299 = traces; 300-399 = slight; 400-499 = small; 500-599 = modest.
[7]Percent boneless, closely trimmed, retail cuts (% BCTRC) = [49.936 – (0.0848 × 2.204 × Hot Carcass Weight, kg) – (4.376 × 0.393 × 12th rib fat thickness, cm) – (3.53 × 0.393 × body wall thickness, cm) + (2.456 × 0.155 × LM area, cm2)].
[8]Spermatozoa concentrations were measured as hundreds of millions per milliliter. The hemocytometer has a counting chamber volume of 1 cubic millimeter. Five large squares were counted for each ejaculate sample, the four corner squares, and the middle square. To calculate the spermatozoa concentration: Total number of sperm counted x dilution factor x hemocytometer factor x conversion factor. The dilution rate was 1:200, the hemocytometer factor was 50, and the conversion factor (converted units to spermatozoa/cubic centimeter, or ml) was 1,000.
[9]Spermatozoa motility score: 1 = no forward movement to 4 = fast forward movement.

Placental development during early pregnancy in sheep: Effects of assisted reproductive technology on fetal and placental growth[1]

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Application of ART techniques decreased fetal size and cell proliferation in fetal and maternal placenta during early pregnancy. Thus, ART may have specific effects on growth and function of ovine maternal and fetal placenta and fetal tissues through regulation of cell proliferation and tissue growth, and likely other mechanisms.

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SUMMARY

Assisted reproductive technologies (**ART**) may have profound effects on placental and fetal development, possibly leading to compromised pregnancy. To determine the effects of ART on the fetal size and cellular proliferation in maternal and fetal placental tissues, pregnancies were achieved through natural breeding (**NAT**), or transfer of embryos generated through in vivo (**NAT-ET**), in vitro fertilization (**IVF**), or in vitro activation (**IVA, clones**). On day 22 of pregnancy, tissues were collected and fetuses were measured. Then, expression of Ki67 (a marker of proliferating cells) was determined using immunohistochemistry followed by image analysis. Fetal length and labeling index (proportion of proliferating cells) in maternal and fetal placenta were less ($P<0.05$) in NAT-ET, IVF and IVF than in NAT. Thus, ART, including simply embryo transfer, may have deleterious effects on growth and function of ovine placental and fetal tissues through regulation of cell proliferation and tissue growth. These data provide a foundation for determining the expression of specific factors regulating placental and fetal tissue growth in pregnancies after ART application. In addition, these data will help us to better understand placental regulatory mechanisms in compromised pregnancies, and to identify strategies for rescuing such pregnancies.

INTRODUCTION

Early pregnancy is a critical period because of the major developmental events that take place, including embryonic organogenesis as well as formation of the placenta, a process known as placentation manifested by enhanced cell proliferation and vascular development (Mossman, 1937, 1987; Green and Winters, 1945; Boshier, 1969; Guillomot et al., 1981; King, 1982; Reynolds et al., 2002, 2006, 2010).

The pattern of placental growth during early pregnancy after natural breeding has been established for sheep (Zheng et al., 1996; Grazul-Bilska et al., 2010, 2011). Comparison of the development of placentas from natural pregnancies and pregnancies achieved by various assisted reproductive technologies (ART), such as after transfer of embryos created through in vitro fertilization (IVF) has demonstrated differences in placental and fetal growth in several species (Barnes, 2000; Cai et

al., 2006; Grazul-Bilska et al., 2006; Romundstad et al., 2006; Allen et al., 2008; Collier et al., 2009; Delle Piane et al., 2010; Sellers Lopez et al., 2010; Esh-Broder et al., 2011; Tomic and Tomic, 2011). For early pregnancy in cows, both greater and less crown-rump length of fetuses created in vitro and then transferred compared to fetuses created in vivo has been reported (Bertolini et al., 2002; Farin et al., 2006). However, data concerning fetal and placental growth including cell proliferation in utero-placental tissues during early pregnancy established through ART application are very limited.

Factors influencing fetal and placental growth have a dramatic impact on fetal and neonatal survival and development (Reynolds and Redmer, 2001; Reynolds et al., 2002, 2006, 2010). Recent observations indicate that compromised fetal growth impacts not only the neonatal period but also life-long health and productivity in humans and livestock species (Nathanielsz 2006, Barker 2007).

We hypothesized that growth of maternal and fetal placenta, and fetus will be altered in pregnancies achieved through application of ART compared to natural pregnancies. In addition to our control group which was naturally bred (NAT), we chose three ART methods to establish pregnancies as follows: (i) superovulation induced by multiple injections of follicle stimulating hormone (FSH) combined with natural breeding, embryo flushing from donors and transfer to recipients (NAT-ET), (ii) transfer of embryos obtained through in vitro fertilization (IVF) of oocytes collected after induction of multiple follicular development using FSH, and (iii) transfer of embryos obtained through in vitro activation (IVA; i.e., parthenotes, which are clones containing only maternal genes) of oocytes collected from FSH-treated donors. In the NAT-ET group, embryos were only briefly removed from uterine environment and had maternal and paternal genomes, in IVF group embryos were created on culture dish and possessed both maternal and paternal genomes, but in IVA group embryos created on culture dish had only maternal genome. Parthenogenetic embryos are used to study the role of maternal genome and the effects of a lack of paternal genome on further embryonic development, imprinted genes and other processes in several species (Loi et al., 1998; Xu and Yang, 2001; Krivokharchenko et al., 2003; Kono et al., 2006; Ferrandi et al., 2002; Lagutina et al., 2004; Grazul-Bilska et al., 2008; Maalouf et al., 2008). The aim of this study was to determine fetal growth and cell proliferation in fetal and maternal placenta during early pregnancy in NAT, NAT-ET, IVF and IVA groups in sheep.

PROCEDURES

Animals and Tissue Collection. The NDSU Institutional Animal Care and Use Committee approved all animal procedures in this study. Estrus was synchronized for adult ewes (n=30; crossbred Western Range, primarily Rambouillet, Targhee, and Columbia) using a CIDR device (MWI, Boise, ID) implanted for 14 days during breeding season. 24 h after CIDR removal, NAT ewes (n=8) were exposed to a fertile ram and naturally bred, but for NAT-ET (n=7), IVF (n=8) and IVA (n=7) groups estrus was checked twice daily using a vasectomized ram. 5%, 86% and 7% of ewes expressed estrus 24, 36 and 48 h after CIDR removal, respectively. Starting on day 13 of the estrous cycle, ewes from NAT-ET group were treated twice daily with FSH for 3 days but ewes from IVF and IVA groups were treated with FSH for 2 days (Stenbak et al., 2001; Grazul-Bilska et al., 2003, 2006; Borowczyk et al., 2006). On day 15 of the estrous cycle, ewes from NAT-ET group were exposed to a fertile ram for 24-48 h, but for IVF and IVA groups, ovaries were collected, oocytes isolated, matured, and then fertilized or activated in vitro as described in detail before (Grazul-Bilska et al., 2003, 2006, 2008; Borowczyk et al., 2006). Briefly, cumulus oocyte complexes (COC) were isolated from follicles $\geq$3 mmm; the average number of collected COC/sheep was 19.3±1.6. For IVF and IVA procedures, oocytes (up to 30 oocytes/0.5 ml in 4-well Nunc culture dish) were incubated overnight in maturation media (TCM199; Sigma, St. Louis, MO, USA) supplemented with 10% fetal bovine serum (FBS), ovine FSH [5 μg/mL; oFSH-RP-1; NIAMDD-NIH, Bethesda, MD, USA], ovine LH [5 μg/mL; oLH-26; NIADDK-NIH], estradiol - 17β [1 μg/mL; Sigma], glutamine [2 mM; Sigma], sodium pyruvate [0.25 mM; Sigma], epidermal growth factor [10 ng/mL; Sigma,] and penicillin/streptomycin [100 units/mL penicillin and 100 μg/mL streptomycin; Gibco, Grand Island, NY, USA]). After denuding oocytes from cumulus cells, half of oocytes from each sheep

was used for IVF and another half for IVA. For IVF, oocytes were cultured in fertilization media in the presence of capacitated frozen -thawed sperm (0.5-1 x 10^6 sperm/ml) for 24 h followed by incubation in culture media till embryo transfer (ET; see below). For IVA, oocytes were incubated for 5 min in TCM199 media containing 2% FBS and ionomycin (2.5 μM; Sigma) followed by 3 h incubation with 6-dimethylaminopurine (DMAP; 2 mM; Sigma). In vitro activated oocytes were then transferred to culture media and incubated till ET (see below).

For NAT-ET group, on day 5 post -mating, embryos were flushed, evaluated under the stereomicroscope, and then transferred to synchronized recipients (3 embryos/ recipient). For IVF and IVA groups, in vitro generated embryos were transferred on day 5 after fertilization or activation to synchronized recipient ewes (3 embryos/recipient) as described by Grazul-Bilska et al. (2003, 2006). On day 22 after mating, fertilization or activation utero-placental tissues were collected. For histology/immunohistochemistry, specimen pins were inserted completely through the uterus and FM at the level of the external intercornual bifurcation to maintain specimen morphology; cross sections of the entire gravid uterus (approximately 0.5-cm thick) were obtained using a Stadie-Riggs microtome knife followed by immersion in formalin or Carnoy's solution and embedding in paraffin. Fetuses were separated from fetal membranes and crown-rump length of each fetus was measured. We choose day 22 for tissue collection, since in our previous experiments, we have demonstrated that on days 20-22, the major changes in cell proliferation, vascularization and expression of angiogenic factors appeared in fetal and maternal placenta for pregnancies achieved through natural breeding (Grazul-Bilska et al., 2010, 2011), and also placentation is already initiated (Igwebuike, 2009).

Immunohistochemistry. Immunohistochemical procedures were described previously (Grazul-Bilska et al. 2010, 2011). Briefly, paraffin-embedded uterine tissues containing FM were sectioned at 4 μm and mounted onto slides. Sections were rinsed several times in PBS containing Triton-X100 (0.3%, v/v) and then were treated for 20 min with blocking buffer [PBS containing normal horse serum (2%, vol/ vol)] followed by incubation with specific primary antibody for Ki67 (a marker of proliferating cells; 1:500; mouse monoclonal; Vector Laboratories, Burlingame, CA, USA) overnight at 4˚ C. Primary antibodies were detected by using secondary anti-mouse antibody coupled to peroxidase (ImPress Kit; Vector Laboratories). Then, the sections stained with Ki67 were counterstained with nuclear fast red (Sigma, St. Lois, MO, USA). Control sections were incubated with normal mouse IgG (4 mg/mL) in place of primary antibody.

Image analysis. For each tissue section, images were taken at 400x magnification, using an Eclipse E600 Nikon microscope and digital camera for 5-10 randomly chosen fields (0.025 mm^2 per field) from maternal placenta containing caruncle (CAR), inter-CAR (ICAR) and fetal placenta (FM), separately. To determine labeling index (LI) in maternal and fetal placenta an image analysis system (Image-Pro Plus, Media Cybernetics, Inc., Bethesda , MD, USA) was used as described previously (Grazul-Bilska et al. 2010, 2011). The LI was calculated as the percentage (%) of proliferating Ki67-positive cells out of the total number of cells in CAR, ICAR and FM tissue area.

Statistical Analysis. Data were analyzed using the general linear models (GLM) procedure of SAS and presented as means ± SEM with the main effect of pregnancy type (SAS Institute 2010). When the F-test was significant ($P<0.05$), differences between specific means were evaluated by using the least significant differences test (Kirk 1982).

RESULTS

The length of the fetus was the greatest ($P<0.0001$) in NAT group, less in NAT-ET, and least in IVF and IVA groups (Fig. 1A). In IVF and IVF groups, length of fetus was approximately 2-fold less than in NAT group (Fig. 1A).

Marker of proliferating cells, Ki67 protein was detected in nuclei of fetal and maternal placenta in all groups (Fig. 2). Labeling index was greater ($P<0.001$) in fetal placenta than in maternal placenta in all groups. In NAT group, LI was 24.5±2.9% and 3.5±0.3% in fetal and maternal placenta, respectively. Labeling index in CAR and ICAR of maternal placenta was similar; therefore data were combined for these two uterine compartments within

each group. In maternal placenta, LI was less ($P<0.001$) in NAT-ET group and least in IVF and IVA groups compared to NAT, and in fetal placenta, LI was less ($P<0.001$) in NAT-ET and IVF groups and least in IVA group compared to NAT (Fig. 1B).

DISCUSSION

Application of ART may have no effects or some negative effects on placental and fetal development or pregnancy outcome in several species including humans, mice, sheep or cows. Compared to in vivo natural fertilization, IVF has been demonstrated to affect embryonic and fetal development, placentation and implantation, placental function and growth, duration of gestation, embryonic loss/survival, appearance of some pathologies, birth weight and others in several species (Barnes, 2000; Bertolini et al., 2002; Cai et al., 2006; Farin et al., 2006; Grazul-Bilska et al., 2006; Romundstad et al., 2006; Allen et al., 2008; Collier et al., 2009; Delle Piane et al., 2010; Sellers Lopez et al., 2010; Esh-Broder et al., 2011; Tomic and Tomic, 2011).

In the present experiment, combination of induction of superovulation with natural breeding and ET (our NET-ET group) decreased fetal size by 15%, but application of IVF or IVA decreased fetal size by more than 50% during early pregnancy. For cows, shorter crown-rump length of fetuses created in vitro compared to fetuses created in vivo has been reported for early pregnancy (Bertolini et al., 2002). On the other hand, Farin et al. (2006) reported that length of bovine embryos produced in vitro almost doubled compared to embryos produced in vivo during early pregnancy; this could lead to large offspring syndrome. Thus, conditions created during superovulation combined with natural breeding and embryo transfer, in vitro fertilization or activation and early embryonic development may have negative effects on fetal growth during early pregnancy.

Cell proliferation in maternal and fetal placenta was decreased by application of ART in our study. Although the LI was approximately 10-fold lower in maternal than fetal placenta, the pattern of changes of LI was very similar in both placental compartments. Placental cell proliferation in pregnancies affected/compromised by application of ART or environmental factors (e.g., maternal nutrition, age or others) has received limited attention. However, decreased LI was observed in placenta of adolescent overnourished ewes, which were also characterized by impaired fetal and placental growth during mid to late gestation (Lea et al., 2005; Redmer et al., 2009). In pregnancy compromised by diabetes, both increased and decreased cell proliferation was observed in placenta in rats (Caluwaerts et al., 2000; Zorn et al., 2011). For diabetic mice, decreased cell proliferation in myometrium during early pregnancy was reported (Favaro et al., 2010). On the other hand, cell proliferation was similar in diabetic and healthy human term placenta (Burleigh et al., 2004). Furthermore, several studies demonstrated high cell proliferation rates in utero-placental tissues during early pregnancy achieved through natural fertilization in humans (Korgum et al., 2006; Kar et al., 2007), sheep (Zheng et al., 1996), cows (Boos et al., 2006; Facciotti et al., 2009), rats (Correia-de-Silva et al., 2004) and monkeys (Blankenship and King, 1994; Wei et al., 2005). Thus, high cell proliferation observed in maternal and fetal placenta in natural pregnancy is decreased during early pregnancy after ART application or compromised by other factors in several species. This likely contributes to impaired fetal and placental growth, and offspring outcome.

In the present study, we have evaluated fetal and maternal placental growth on day 22 of pregnancy only. Therefore, we cannot exclude that the differences in placental growth among investigated pregnancy types may decrease due to possible compensatory mechanisms, or alternatively may increase as pregnancy progresses. Thus, future studies should evaluate placental growth during later stages of pregnancy.

Tissue growth including cell proliferation is regulated by growth and other regulatory factors in placenta and other tissues (Zheng et al., 1996; Reynolds et al., 2006, 2010; Grazul-Bilska et al., 2010, 2011). Since we have observed reduced expression of several growth factors known to regulate placental function including fibroblast growth factor (FGF) 2, FGF receptor, placental growth factor and others in maternal or fetal placenta after application of ART during early pregnancy (Johnson et al., 2011), we hypothesize that application of ART decreased expression of regulatory factors which in turn contributed to

reduced cellular proliferation and fetal size. However, the role and expression of factors controlling tissue growth and cell proliferation in placental function requires further investigation.

In summary, application of ART techniques decreased fetal size and cell proliferation in fetal and maternal placenta during early pregnancy. Thus, ART may have specific effects on growth and function of ovine maternal and fetal placenta and fetal tissues through regulation of cell proliferation and tissue growth, and likely other mechanisms. These data provide a foundation for determining the expression of specific factors regulating placental and embryonic tissue growth in pregnancies after ART application. In addition, these data will help us to better understand placental regulatory mechanisms in compromised pregnancies, and to identify strategies for rescuing such pregnancies.

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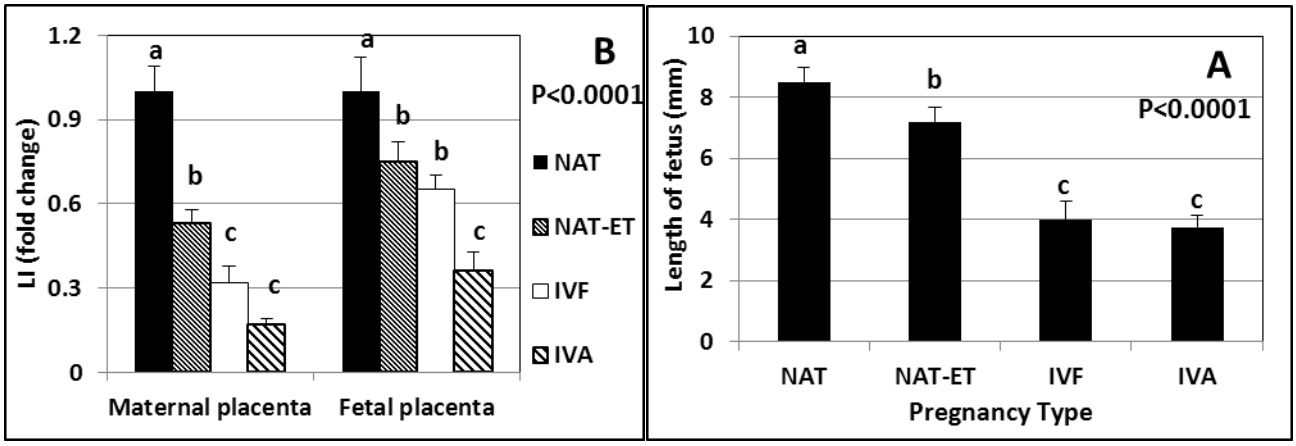

Figure. 1. The length of fetus (A) and labeling index (LI) in maternal and fetal placenta (B) in NAT, NAT-ET, IVF and IVA groups. Values ± SEM with different superscripts (a, b, c) differ within measurement. For LI, data are expressed as fold change compared to NAT control arbitrary set as 1. In NAT group, LI was and 3.5±0.3% and 24.5±2.9% in maternal and fetal placenta, respectively.

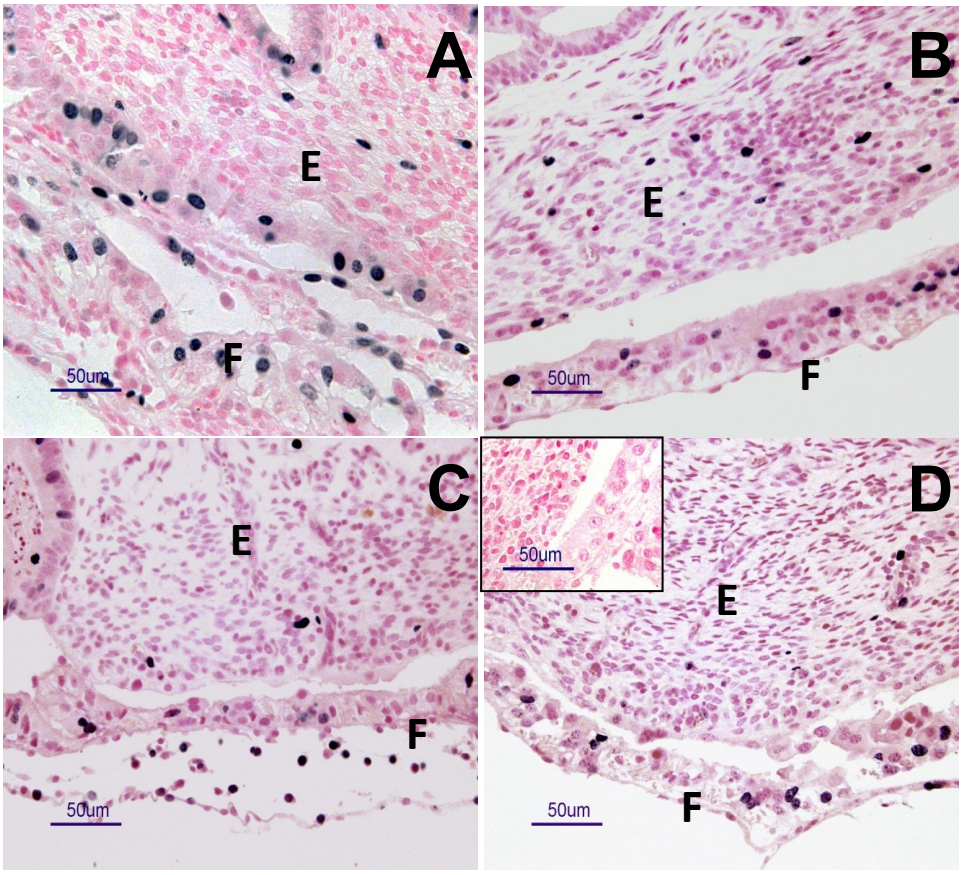

Figure. 2. Representative photomicrographs of immunohistochemical staining for Ki67 in maternal and fetal placenta in in NAT (A), NAT-ET (B), IVF (C) and IVA (D) groups. Dark color represents positive staining and pink color (nuclear fast red staining) indicates unlabeled cell nuclei. Note nuclear staining of Ki-67 in fetal placenta (FM) and endometrium (E, maternal placenta). In inset (D), note a lack of positive staining in the control sections in which mouse IgG was used in place of the primary antibody

Impacts of supplemental arginine on reproductive performance in sheep

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The objective of this study was to determine the effects oral and injectable supplementation of arginine two weeks post breeding on ewe reproductive performance and lamb growth. Prenatal lamb loss accounts for a large portion of economic loss in the sheep industry. Sheep producers could benefit from a supplementation protocol that recovered these losses.

INTRODUCTION

Reproductive performance is the largest determinant of income in livestock production. In the U.S. sheep industry, embryonic and fetal death can account for 25-50% of the total number of ovulations (Knights, et al., 2003; Dixon et al., 2007). The majority of embryonic loss occurs before d 18 of gestation (Hulet et al., 1956; Moore et al., 1960; Quinlivan, 1966). However, the loss of individual embryos can occur without a complete loss of pregnancy, such as in the case of multiple pregnancies (Rhind et al., 1980; Schrick and Inskeep, 1993). In sheep, it has been reported that 30% of fertilized ova are not represented by live births, resulting in frequent, but unrecognized economic loss (Knights et al., 2003; Bolet, 1986; Dixon et al., 2007). Furthermore, a small percentage of embryos are inherently non-viable (Wilmut et al., 1986), which suggests early embryonic loss is likely preventable in the ewe. Strategies to enhance prenatal growth and survival could clearly have a major economic impact in the sheep industry. Past research by NDSU has shown that supplemental arginine (**Arg**) can recover embryonic and/or fetal loss in fall lambing ewes synchronized to estrus with exogenous hormones (Saevre et al., 2011; Luther et al., 2009).

The amino acid L-arginine is a precursor for nitric oxide and important in the synthesis of polyamines and proteins, all of which are essential to proper development of the embryo and placenta. Past NDSU research has observed increased pregnancy rate in ewes treated with injectable L-arginine when compared to control ewes by 45% (Saevre et al., 2011; Luther et al., 2009). However, this previous research has always utilized an injectable Arg source, which is not readily adaptable to producer use. In order for producer acceptance of Arg supplementation to occur, a feed option must be found. It is reasonable to hypothesize from the previous studies that supplementation of rumen-protected arginine would have beneficial impacts on prenatal growth and survival for ruminant livestock.

The objective of this study was to determine the effects of injectable (Exp. 1) and oral (Exp. 2) Arg supplementation provided two wk post breeding on reproductive performance of naturally stimulated fall lambing ewes.

[1]Partial support for this research was provided by the North Dakota State Board of Agricultural Research and Education Research Fund – Animal Agriculture and Hay Committee. The authors would like to thank David Pearson, Erin Gaspar, Donald Drolc, and Don Stecher for their assistance in conducting this trial.

PROCEDURES

All procedure were approved by the Animal Care and Use Committee of North Dakota State University. This study was conducted at the Hettinger Research Extension Center in Hettinger, ND.

Animals and Diets. Rambouillet ewes of a similar BW (142.6 ± 15.01 lbs.) were randomly assigned to one of six treatment groups: control (**CON**; n= 25), IV -alanine (**IVALA**; n=20), IV-arginine (**IVARG**; n=23), rumen-protected arginine (**RPARG**; n=20), soybean meal (**SBM**; n=23), fishmeal (**FM**; n=24). Ewes were exposed to 15 fertile ram lambs for 2 weeks before the trial start. During this time, ewes were fed one pound of corn/hd/d. Ewes were exposed to fishmeal at 12% of corn intake for 4 days during the 2 weeks pre-breeding to adapt them to the taste and smell of fishmeal. Ewes were fed 6 lb per day (as fed) a ration consisting of 25% alfalfa haylage and 75% grass hay. Ewes were exposed to mature rams one day before the start of the trial. Any ewes that were bred during the two weeks before the start of the project were removed from the trial. Ewes that received breeding marks 10 - 17 d post ram introduction were allocated to treatments. Thereafter, ewes were moved to a different pen and exposure to fertile rams for an additional 14 days. From d 0 (estrus) to d 14 (post estrus) ewes received their assigned treatment. In Exp. 1, all ewes received 1 lb of corn daily and injected with similar volumes of their treatment to provide 30 $mg \cdot kg^{-1} \cdot hd^{-1} \cdot d^{-1}$ Arg. Intravenous injections of arginine,alanine, and saline were administered daily to IVARG, IVALA, and CON ewes. In Exp. 2, all ewes received 1 lb/d of their respective treatments to provide 30 $mg \cdot kg^{-1} \cdot hd^{-1} \cdot d^{-1}$ Arg to the Arg supplemented treatments. Treatments were: RPARG (0.15 g/kg BW rumen protected product mixed with ground corn), SBM (25:75 soybean meal: corn), and FM (37.5:62.5 fishmeal: corn). The CON treatment form Exp. 1 served as the control treatment for Exp. 2. Blood samples were collected from 12 ewes per treatment group prior to administration of treatment every other day during 14-day trial treatment period. Blood samples were assayed for concentrations of progesterone. At lambing, birth weight, birth type, and sex were collected. Weaning weights were collected when the average age of lambs was 60 d.

Statistical Analysis. Pregnancy, prolificacy, and lambing rates were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). Arginine treatment served as the fixed effect. The fixed effect of day was utilized in the REPEATED measures analysis for progesterone concentrations. The model included the fixed effects of dietary treatment, day, and treatment x day. Significance was determined at $P \leq 0.05$. To partition day effects and treatment × day interactions, LS Means were utilized ($P \leq 0.05$).

RESULTS

In Exp. 1, no differences were detected for pregnancy, prolificacy, and lambing rates among treatments ($P = 0.95$, 0.35, and 0.70, respectively; Table 1). Similary, in Exp. 2, no differences were detected for pregnancy, prolificacy, and lambing rates among treatments ($P = 0.94$, 0.61, and 0.80, respectively; Table 2). Additionally, there were no differences detected for progesterone concentrations for treatment or treatment by day interactions among treatments in Exp. 1 or 2 ($P = 0.58$ and 0.34, respectively; Figures 1 and 2, respectively). There was a day effect for both Exp. 1 and 2, but this observation was expected ($P < 0.0001$) due to the estrous cycle. Similar to gestational performance, there were no differences detected for birth weights in Exp. 1 and 2 among treatments ($P = 0.57$; $P = 0.73$, respectively; Tables 1 and 2). In Exp. 1, male lamb birth weights were significantly higher than female lambs ($P = 0.014$; data not shown). However, birth weights for Exp. 2 were similar for all treatments. Prolificacy had no effect on lamb birth weight in Exp. 1 ($P = 0.07$), but for Exp. 2 single born lambs were significantly heavier than twin lambs ($P < 0.0001$; data not shown). There were no differences detected for weaning weights in Exp. 1 and 2 among treatments ($P = 0.53$; $P = 0.57$, respectively; Tables 1 and 2). In Exp. 1, no differences were detected in weaning weights among the birth type ($P = 0.17$), however, in Exp. 2, single born lambs were significantly heavier than twin born lambs ($P = 0.04$; data not shown). In Exp. 1, male lamb weaning weights were significantly higher than female birth weights ($P = 0.05$; data not shown), but in Exp. 2 no differences were seen between male and female lamb weaning weight ($P = 0.92$).

DISCUSSION

In the present study, pregnancy, prolificacy, and lambing rates

were not influenced through injectable or oral treatments. In contrast, research from this laboratory reported greater pregnancy rates in ewes supplemented with injectable Arg from d 0 through 14 post breeding and also ewes supplemented d 9 through 14 post breeding (Luther et al., 2009; Saevre et al., 2011). Moreover, pregnancy rates were much lower in the previous studies than in our study. Pregnancy rates were as follows: ARG (55%) vs. CON (60%), ARG (55%) vs. CON (30%), and IVARG (88%) vs. CON (88%) vs. RPARG (86%) (Luther et al., 2009; Saevre et al., 2011). We hypothesize that the differences in pregnancy rates between these projects could be due to a difference in reproductive synchronization models utilized as a comparison. Ewes in the previous two studies were synchronized artificially with a CIDR and an injection of PG-600, whereas the ewes in the present study were naturally synchronized using ram exposure.

Arginine is important for many biological functions, including the synthesis of nitric oxide (Gouge et. al., 1998; Manser et. al., 2004). Other studies have hypothesized that treatment with arginine at or slightly before the time of maternal recognition of pregnancy in the ewe may have enhanced the survival of the embryo during early embryogenesis through its role in polyamine and nitric oxide synthesis (Luther, et al., 2009). In the current study, however, increased pregnancy, prolificacy or lambing rates were not observed for arginine treated ewes.

As stated previously, arginine is important for the synthesis of nitric oxide, which is important for dilating blood vessels, therefore increasing tissue blood flow. Increases in ovarian blood flow or flow to the corpus luteum during early pregnancy could result in higher progesterone concentrations. This could result in a more ideal environment for early embryonic survival in arginine treated ewes. However, no differences in progesterone concentrations were observed between arginine treated ewes and the controls.

IMPLICATIONS

Although previous results imply that embryonic survival in sheep can be enhanced when supplemented with arginine, we did not detect any improvements in reproductive performance or lamb growth in ewes supplemented with either injectable or rumen-protected forms of arginine. We hypothesize that supplemented arginine might enhance reproductive performance in compromised models, such as the previous studies (chemical synchronization, etc.). However, further research is needed to develop this hypothesis.

Table 1. Effects of daily injection of treatments[1] two weeks post breeding on pregnancy, prolificacy and lambing rate in sheep

Item	Control	IV-Alanine	IV-Arginine	SEM	*P*-value[2]
Pregnancy[3]	88	91	88	7.1	0.95
Prolificacy[4]	1.32	1.21	1.43	0.11	0.35
Lambing Rate[5]	1.16	1.10	1.25	0.13	0.70
Birth Weight	11.8	12	11.5	0.53	0.57
Weaning Weight	54.3	48.7	50.6	3.0	0.53

[1]Control, 7 mL/kg BW saline (n=25); IV-Alanine, 0.110 mL/kg of BW (n=20), IV-Arginine, 0.093 mL/kg of BW, (n=23).
[2]*P*-value for F test of the mean.
[3]Pregnant treated ewes that lambed to the first estrus.
[4]Lambing rate of ewes that lambed.
[5]Lambing rate of ewes treated.

Table 2. Effects of daily injection of treatments[1] two weeks post breeding on pregnancy, prolificacy and lambing rate in sheep

Item	Control	RPARG	FM	SBM	SE	*P*-value[2]
Pregnancy[3]	88	86	89	83	8	0.94
Prolificacy[4]	132	117	117	126	10	0.61
Lambing Rate[5]	1.16	1.00	1.04	1.04	0.12	0.80
Birth Weight	11.8	11.6	11.6	11.4	0.57	0.73
Weaning Weight	55.0	56.7	51.3	52.7	3.0	0.57

[1]Control, 7 mL/kg BW saline (n=25); RPARG, 0.15 g/kg BW (n=20); FM, 25:75 ration (FM:corn) (n=24); SBM, 37.5:62.5 ration (SBM:corn) (n=23).
[2]*P*-value for F test of the mean.
[3]Pregnant treated ewes that lambed to the first estrus.
[4]Lambing rate of ewes that lambed.
[5]Lambing rate of ewes treated.

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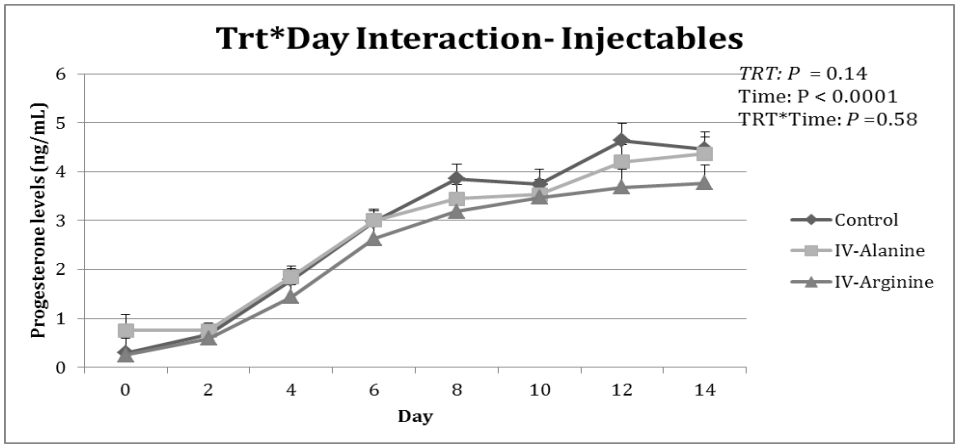

Figure 1. Progesterone concentrations throughout the treatment period in arginine and alanine injected ewes. Data are means ± S.E.

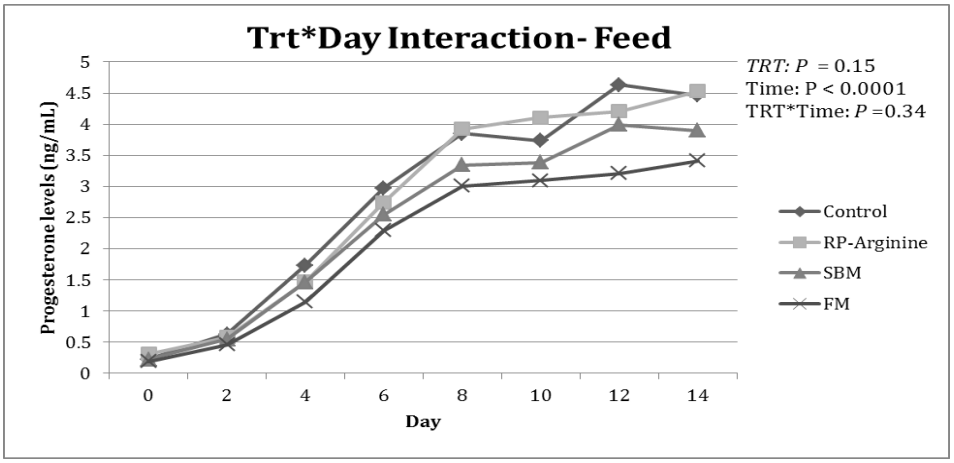

Figure 2. Progesterone concentrations throughout the treatment period in rumen-protected arginine (RPARG), fishmeal (FM), and soybean meal (SBM) treated ewes. Data are means ± S.E.

Efficacy of pregnancy specific protein B assay to predict pregnancy and pregnancy rate in sheep

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Our objective was to evaluate the effectiveness of the pregnancy specific protein B assay to predict early pregnancy and pregnancy rate in sheep. This test accurately detected flock pregnancy status after 30 days of pregnancy. Multiple pregnancies had greater PSPB concentrations in two of the four breeds.

INTRODUCTION

Most North Dakota sheep production scenarios require substantially more resources, such as feed, labor, and facilities during late gestation and lactation. Early identification of pregnancy and pregnancy rate in sheep provides managers several options to increase flock productivity. First, removal of non-pregnant ewes can increase available resources for pregnant ewes. Second, identification of pregnancy status of ewe lambs (9 months of age) allows producer to market ewe lambs as lamb, instead of waiting until after the lambing season, when ewe lambs will be older than 12 months and are more likely to be classified as mutton. Third, identification of pregnancy rate in sheep allows producer to target feed resources to ewes baring multiple lambs. Twin baring ewes require 30% more feed than singleton baring ewes. Finally, identification of early and late lambing ewes allows producer to target feed resources to ewes during period of greatest need. Late gestation singleton and twin baring ewes require 50 and 80% more feed, respectively, than ewes in early gestation.

Ultrasonic imaging is the most common method to determine pregnancy and pregnancy rate. This technique requires expensive equipment and a highly trained technician. Many states require this technique be conducted by a licensed veterinarian. Qualified technicians or veterinarians are not available to many sheep producers or costs associated with travel to remote locations are prohibitive. BioTracking, LLC developed a commercially available pregnancy-specific protein B (PSPB) test for pregnancy in cattle. The test was named BioPRYN®, with PRYN standing for "Pregnant Ruminant Yes No". This technology was licensed by BioTracking from the University of Idaho and the assay was converted to an enzyme-linked immunosorbent assay (ELISA). The PSPB test was later developed for sheep and goats.

Our objectives for this study were to determine the earliest day of pregnancy that the BioPRYN test could accurately detect pregnancy status and pregnancy rate.

PROCEDURES

All experimental protocols were approved by the North Dakota State University Animal Care and Use Committee. This study

consisted of two different experiments both held at the NDSU Sheep Unit in Fargo, ND.

In Exp. 1, Columbia and Hampshire ewes were exposed to intact rams equipped with marking harnesses on August 15th, 2011. Breeding marks were identified and recorded. On days 20, 25, 30, 40, and 60 post-breeding, blood samples were collected to determine PSPB concentrations. In Exp. 2, Dorset and Katahdin ewes were exposed to intact rams on September 27, 2011. Blood samples were taken from all ewes 7, 9, and 11 weeks post ram introduction. Lambing records were used to verify conception dates and lambing rates.

All blood samples were collected via jugular venipuncture into 10 mL serum tubes (BD Vacutainer Serum, Becton, Dickinson and Company, Franklin Lakes, NJ) and immediately placed on ice. Samples were centrifuged at 4˚C for 30 min at 1,500 x g and serum was transferred into plastic 2.0 mL microcentrifuge tubes and frozen at -20 ˚C until assayed. After all samples were collected, samples were shipped to Bio-Tracking for analysis.

Only, 2 Columbia ewes and 1 Hampshire ewe gave birth to triplets. Two Katahdin ewes gave birth to singleton lambs. All five ewes were removed from the analysis because of limited number of ewes within lambing groups.

Pregnancy classification as determined by BioPRYN at different stages of pregnancy was analyzed using the PROC FREQ and CHI-SQUARE function of SAS (SAS Inst., Inc., Cary, NC). Repeated measures of the MIXED procedure of SAS were used to analyze serum concentrations of PSPB. Breed, day of pregnancy, and their interaction were significant in the model; therefore, breed and day of pregnancy sorted and analyzed independently. Data are presented as least squares means and treatment differences were considered significant at $P \leq 0.05$.

RESULTS

To determine the earliest that the BioPRYN test could detect pregnancy, samples were classified into three subcategories: less than 25, between 25 and 30, and over 30 days of pregnancy. There was a significant ($P < 0.01$) interaction between day of pregnancy tested and classification of pregnancy via BioPRYN testing. The test accurately detected pregnancy in 2, 83, and 98% of pregnant ewes when tested less than 25, between 25 and 30, or greater than 30 days of pregnancy, respectively (Table 1).

Concentration of PSPB are presented for Columbia ewes that lambed to singleton and twin pregnancies (n = 14 and 7, respectively; Table 2). Number of lambs born did not have an effect on PSPB concentrations on days 20 and 60 post breeding ($P \geq 0.27$) in Columbia ewes. On days 25, 30, and 40 post breeding, twin pregnancies had greater ($P \leq 0.05$) PSPB concentrations than singleton pregnancies.

Concentration of PSPB are presented for Hampshire ewes that lambed to singleton and twin pregnancies (n = 11 and 7, respectively; Table 3). Number of lambs born did not have an effect on PSPB concentrations on days 20, 25, 30, 40, and 60 post breeding ($P \geq 0.06$) in Hampshire ewes.

Concentration of PSPB taken 49, 53, and 67 days post ram introduction to Dorset ewes that lambed to singleton, twin, and triplet pregnancies (n = 21, 37, and 5, respectively; Table 4). Actual days of pregnancy for the blood samples were back calculated from the lambing date. The average actual days of pregnancy were 40, 54, and 68 ± 7.5 for the three respective sampling dates. Number of lambs born did not have an effect on PSPB concentrations taken 49 days post ram introduction ($P = 0.11$) in Dorset ewes. Twin pregnancies had greater ($P \leq 0.01$) PSPB concentrations than singleton pregnancies on days 53 and 67 post ram introduction. Triplet pregnancies were not different ($P \geq 0.13$) from twin or singleton pregnancies on days 53 and 67 post ram introduction.

Concentration of PSPB taken 49, 53, and 67 days post ram introduction to Katahdin ewes that lambed to twin and triplet pregnancies (n = 13 and 6, respectively; Table 5). Actual days of pregnancy for the blood samples were back calculated from the lambing date. The average actual days of pregnancy were 37, 51, and 65 ± 8.1 for the three respective sampling dates. Number of lambs born did not have an effect on PSPB concentrations taken 49 days post ram introduction ($P \geq 0.06$) in Katahdin ewes.

DISCUSSION

Our first objective was to determine when the assay could accurately detect pregnancy in sheep.

Although pregnancy was detected as early as day 20 in one ewe, 72% of pregnant ewes were falsely determined to be open when the test was conducted before 25 days of pregnancy. Testing between day 25 and 30 of pregnancy accurately determined 83% of pregnancies; however, the remaining ewes were determined to be open or required additional testing. Testing for PSPB after 30 days of pregnancy accurately identified 98% of pregnant ewes. One ewe tested open at 30 days of pregnancy; however, the next test identified the ewe as pregnant. Although this study was not designed to identify false positives, we did not have any open ewes that were determined to be pregnant by the PSPB test. There were some ewes that tested pregnant on initial tests but later testing identified the ewe to be open. We hypothesize that this was a result of failed pregnancies.

In agreement with previous research (Willard et al., 1995), breed and age of pregnancy were strongly correlated with PSPB concentrations. These contributing factors make it difficult to randomly take a sample from ewes that have been exposed to rams and determine pregnancy rate. Concentrations of PSPB were not correlated to litter size in the Hampshire and Katahdin breeds; whereas, concentration of PSPB and litter size were correlated in the Columbia and Dorset breed. There may be opportunity for sheep producer of this breed to sort into pregnancy groups by PSPB concentration; however, exact date of breeding must be known. Unfortunately, triplet baring ewes from both the Dorset and Katahdin breed did not express greater PSPB concentration than twin baring ewes.

Although, PSPB testing cannot definitely identify pregnancy rate, there may be opportunity for sheep producers to improve efficiency through this test. First, identification of pregnancy beyond 60 days of gestation was 100% accurate; therefore, ewes that failed to become pregnant or lost a pregnancy can be identified and removed from the flock. Second, individual ewe nutrition and management requirements increase as pregnancy progresses and if multiple pregnancies are present. Similarly, within a breeding group PSPB concentration were higher in ewes that possessed the oldest pregnancies or multiple pregnancies. Therefore, sorting a group of ewe by PSPB concentration would allow for producers to improve efficiency of feed, labor and facility resources.

IMPLICATIONS

The PSPB test was very effective at detecting pregnancies beyond 30 days and it is very likely to be as good as or better than most ultrasound technicians. Although PSPB concentrations were able to differentiate between single and multiple pregnancies, it was not consistent between breeds and age of pregnancy must be known. Skilled ultrasound technicians would likely be more accurate at detection of pregnancy rate than the PSPB test.

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Table 1. Number and percent of pregnant ewes classified as open, recheck or pregnant by PSPB test at different days of pregnancy in Exp. 1 and 2

	BioPRYN Classification[1]			
Days of Pregnancy	Open	Recheck	Pregnant	*P*-Value
< 25 days	31(72)	11(26)	1(2)	<0.01
25 – 30 days	2(3)	11(14)	67(83)	<0.01
≥ 30 days	1(0.3)	7(2)	299(98)	<0.01

[1]BioPRYN classification are open (less than 15 ng/mL), retest (15 to 30 ng/mL), or pregnant (greater than 30 ng/mL).

Table 2. Serum pregnancy specific protein B (PSPB) concentrations in pregnant Columbia ewes[1]

	Number of Lambs Born				
Day of Pregnancy	Singleton	Twin	Triplet	SE	*P*-Value
20	11.6	14.5	—	1.9	0.24
25	33.1[a]	46.2[b]	—	5.0	0.05
30	59.7[a]	80.3[b]	—	6.1	<0.01
40	71.8[a]	90.8[b]	—	4.9	<0.01
60	114.2	125.5	—	10.5	0.13

[1] n = 14 and 7 for singleton and twin pregnancies, respectively.

Table 3. Serum pregnancy specific protein B (PSPB) concentrations in pregnant Hampshire ewes

	Number of Lambs Born[1]				
Day of Pregnancy	Singleton	Twin	Triplet	SE	*P*-Value
20	14.1	12.7	—	3.4	0.76
25	34.5	36.1	—	5.1	0.82
30	56.3	52.7	—	8.2	0.74
40	55.9	69.5	—	5.6	0.06
60	72.9	86.2	—	11.8	0.38

[1] n = 11 and 7 for singleton and twin pregnancies, respectively.

Table 4. Serum pregnancy specific protein B (PSPB) concentrations in pregnant Dorset ewes

	Number of Lambs Born[1]				
Days after Ram Introduction[2]	Singleton	Twin	Triplet	SE	*P*-Value
49	64.4[a]	79.2[b]	69.9[ab]	11.3	0.11
53	77.4[a]	97.5[b]	93.2[ab]	9.3	<0.01
67	79.7[a]	98.4[b]	89.1[ab]	9.4	<0.02

[1] n = 21, 37, and 5 for singleton, twin, and triplet pregnancies, respectively.
[2]The average days of pregnancy were 40, 54, and 68 ± 8.1 for the three respective sampling dates.

Table 5. Serum pregnancy specific protein B (PSPB) concentrations in pregnant Katahdin ewes

	Number of Lambs Born[1]				
Days after Ram Introduction[2]	Singleton	Twin	Triplet	SE	*P*-Value
49	—	66.4	74	6.3	0.35
53	—	67.5	84	9.3	0.17
67	—	65.7	77.7	7.8	0.22

[1] n = 13 and 6 for twin triplet pregnancies, respectively.
[2]The average days of pregnancy were 37, 51, and 65 ± 8.1 for the three respective sampling dates.

Effects of maternal metabolizable protein supplementation during the last 50 days of gestation on ewe performance and offspring performance from birth to weaning[1]

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The objectives of this trial were to determine the effects of maternal metabolizable protein supplementation during the last 50 days of gestation on ewe performance and offspring performance. Results indicate dam performance can be positively impacted by supplementing MP at or above requirements through maintaining dam BW and BCS, buy may have marginal effects on lamb performance from birth through weaning.

INTRODUCTION

Crude protein supplementation not only allows dams to maintain BW and BCS, but appears to improve offspring performance (Stalker et al., 2006; Larson et al., 2009). Crude protein supplementation to the dam is just one method of improving livestock performance during gestation. Metabolizable protein has been defined as the protein and amino acids that are digested and absorbed post-ruminally (Burroughs et al., 1975). Since MP is the protein directly available to the dam, it may be an indicator of how protein intake during gestation will ultimately affect offspring performance between birth and weaning. However, there has been minimal research conducted on the effects of MP intake during late gestation in sheep on offspring performance.

We hypothesized that the greater proportion of the diet that is composed of MP would yield improved offspring growth by potentially increasing nutrient transfer by the placenta or by increased nutrients within the milk. Therefore, the objectives were to evaluate isocaloric diets with increasing levels of MP during late gestation on ewe performance and offspring growth.

PROCEDURES

All procedures were approved by the NDSU Animal Care and Use Committee. This study was conducted at the Hettinger Research Extension Center in Hettinger, ND.

Ewes. On d 99 and 100 of gestation, in two consecutive years, ewes were weighed and body condition scored. On d 100 ± 8 (SD) of gestation, using the average of the initial weights (d 99 and 100 of gestation), ewes were stratified by BW, BCS, age, and expected lambing date to one of three isocaloric dietary treatments (Table 2; n = 7): **100MP1**: 100% of the MP requirements on a DM basis during the last 4 weeks of gestation of a ewe carrying twins (NRC, 2007); **60MP1**: 60% of 100MP1; and **80MP1**: 80% of 100MP. Isocaloric dietary treatments (Table 2; n = 4) in year 2 were: **60MP2**: 60% of MP requirements; **100MP2**: 100% of the MP requirements; and **140MP2**: 140% of MP requirements on a DM basis of a ewe carrying twins during the last 4 weeks gestation (NRC, 2007). Once ewes had lambed, the ewes and lambs were intermingled between dietary treatments where they were maintained on a lactation ration (Table 1).

[1]This project was supported by National Research Initiative Competitive Grant no. 2009-35206-05276 from the USDA National Institute of Food and Agriculture. The authors would like to thank David Pearson, Donald Drolc, Donald Stecher, Tammi Neville, and James Kirsch for their assistance in conducting this trial.

Lambs. In both years, lambs were weighed and tagged within 24 h of birth; sex, lambing assistance, and lamb vigor were also recorded. Lambs were then moved with the ewes to grouping pens and had full-access to creep pellet and water prior to weaning. At 14 days of age and at weaning all lambs were vaccinated for tetanus and *Clostridium Perfringens* types C and D (CD-T; Bar Vac CD-T, Boehringer Ingelhein, Ridgefield, CT), tails were docked, and ram lambs were castrated. Lambs were weaned at 69 ± 5 d (SD) of age in year 1 and at 61 ± 12 (SD) d of age in year 2 and weighed.

Statistical analysis. Ewe performance and lamb performance were analyzed utilizing the MIXED procedures of SAS (SAS Inst. Inc., Cary, NC). When a significant *F*-test was observed ($P \leq 0.15$), pre-planned comparisons of linear and quadratic contrasts were utilized to partition treatment effects. Significance was set at $P \leq 0.05$ and tendencies at $P \leq 0.10$.

RESULTS

Year 1

Ewes. Ewe weight change at lambing, change in BCS during gestation, gestation length, and lamb birth weight per unit initial or final ewe BW were not affected ($P \geq 0.22$; Table 3) by maternal dietary treatment. As MP increased in the diet, there was a linear ($P = 0.01$; Table 3) increase in change in BW during gestation. At lambing, ewe BW ($P = 0.02$) and BCS ($P = 0.01$) increased linearly as MP in the diet increased. There was a linear ($P < 0.001$) reduction in the percentage of BW loss from d 100 of gestation to immediately postpartum as MP increased in the diet. There was a linear increase in BCS loss as MP was reduced in the diet from d 100 ($P = 0.01$; Table 3) and 142 ($P = 0.05$) of gestation to immediately postpartum.

Lambs. There was no effect ($P = 0.30$; Table 3) of maternal dietary treatment on lamb birth weight. There tended to be a linear increase ($P \geq 0.08$) in weaning BW and ADG from birth to weaning as MP intake increased in the diet.

Year 2

Ewes. There were no significant effects ($P \geq 0.35$; Table 4) of maternal dietary treatment on ewe weight change from d 142 to lambing, change in BCS during gestation, change in BCS from d 100 and 142 to lambing, or BCS at lambing. As MP increased in the diet, there was a linear ($P = 0.01$) increase in change in BW. There was a linear ($P < 0.001$) reduction in percent BW loss from d 100 of gestation to immediately postpartum as MP increased in the diet. At lambing, ewe BW increased ($P = 0.02$) linearly as MP in the diet increased.

Lambs. There were no effects ($P \geq 0.25$; Table 4) of maternal dietary treatment on lamb birth BW, weaning BW, age at weaning, percent BW growth from birth to weaning, and ADG from birth to weaning.

DISCUSSION

Previous research has indicated that supplementation of protein during late gestation improves dam BW gain and BCS and restriction of protein results in reductions of dam BW and BCS (Stalker et al., 2006). Along with the current study, these studies suggest that increasing CP intake during late gestation enhances dam performance and minimizes the mobilization of dam body reserves to maintain fetal growth.

Similar to our results, Anthony et al. (1986) did not observe any effects on calf birth weight when dams were either fed low (81%) or high (141%) protein diets during late gestation (89 days prior to parturition). Amanlou et al. (2011) also observed no effects of maternal MP supplementation on lamb birth weight. Overall, our results suggest that birth weight may not be negatively impacted by maternal MP restriction.

Average daily gain of lambs from birth to weaning has also been positively affected by maternal nutrition during late gestation. Calves born to cows supplemented with CP during late gestation had increased ADG from birth to weaning compared with those calves born to unsupplemented cows (Stalker et al., 2006). In year 1, weaning BW tended to increase as MP increased in the diet, but weaning BW was not altered by maternal MP intake in year 2. The current results suggest that weaning weights may be reduced in lambs born to dams fed less than required MP, but feeding above MP requirements during late gestation may not improve weaning weights of lambs.

IMPLICATIONS

These results suggest that dam performance can be positively impacted by supplementing MP at or above requirements through maintaining dam BW and BCS. Restricting MP during late

gestation may not negatively impact lamb birth weights, but may reduce weaning weights especially when ewes are below a BCS of 3. However, supplementing above MP requirements during late gestation will likely improve weaning weights. The results of the current study suggest that supplementing MP during late gestation may be a key asset to be utilized to improve dam performance from late gestation to weaning, but may have marginal effects on lamb growth from lambing to weaning.

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Table 1. Nutrient composition of fescue straw and lactation ration in years 1 and 2

	Fescue Straw[1]		
Item	Year 1	Year 2	Lactation Ration[2]
Diet, % DM	64.51	56.51	100.00
DM, %	83.05	77.61	64.37
NEm, Mcal/kg	2.22	2.12	—
CP, % of DM	3.04	3.07	11.52
MP, % of DM	1.95	1.97	—
NDF, % of DM	79.85	81.13	48.05
ADF, % of DM	48.97	51.10	27.16
Ash, % of DM	9.49	7.78	8.59

[1]Ewes were fed fescue straw in each year to limit metabolizable protein intake.

[2]Ewes were fed a common ration during lactation across all dietary treatments; 28.5% oats, 28.5% haylage, 42.9% chopped hay.

Table 2. Ingredient and nutrient composition of dietary supplements fed to ewes in year 1 and 2

	Year 1[1]			Year 2[2]		
Item	60MP1	80MP1	100MP1	60MP2	100MP2	140MP2
Ingredient, % DM						
Corn	18.50	15.00	5.00	30.00	19.00	—
DDGS[3]	7.00	20.00	30.00	4.00	24.00	43.00
Soyhulls	9.50	—	—	9.00	—	—
Trace mineral[4]	0.49	0.49	0.49	0.49	0.49	0.49
Nutrient composition						
DM, %	88.75	89.34	89.68	88.64	90.19	92.16
NEm, Mcal/kg	2.00	2.22	2.14	2.05	2.19	2.06
CP, % of DM	13.16	20.21	25.13	10.21	18.67	28.68
MP, % of DM	8.41	13.01	16.31	6.54	11.96	18.37
NDF, % of DM	31.03	30.73	39.79	29.64	31.40	45.34
ADF, % of DM	15.69	7.45	10.49	13.87	8.68	13.34
Ash, % of DM	3.22	3.48	4.55	3.53	3.80	5.13

[1]Maternal diets (DM basis) were balanced for mature ewes baring twins during the last 4 weeks of gestation according to NRC (2007). Treatments: 60MP1: 60% of metabolizable protein of 100MP1; 80MP1: 80% of the metabolizable protein 100MP1; and 100MP1: 100% of the metabolizable protein requirement.

[2]Maternal diets (DM basis) were balanced for mature ewes baring twins during the last 4 weeks of gestation according to NRC (2007). Treatments: 60MP2: 60% of metabolizable protein of 100MP2; 140MP2: 140% of the metabolizable protein of 100MP2; 100MP2: 100% of the metabolizable protein requirement.

[3]Dried distillers grains with solubles

[4]Trace mineral content: 16.0-17.0% Ca; 8.0% P; 21.0-23.0% Salt; 2.75% Mg; 3 ppm Co; 5 ppm Cu; 100 ppm I; 1,400 ppm Mn; 20 ppm Se; 3,000 ppm Zn; 113,500 IU/kg Vitamin A; 11,350 IU/kg Vitamin D; and 227 IU/kg Vitamin E.

Table 3. Effects of maternal metabolizable protein supplementation during the last 50 d of gestation on ewe and offspring performance from birth to weaning for year 1

	Dietary Treatment[1]					Orthogonal Contrasts[4]	
Item	60MP1	80MP1	100MP1	SEM[2]	*P* – value[3]	Linear	Quadratic
Initial BW, lb	139.8	142.0	142.4	2.36	0.71	0.43	0.79
BW at lambing,[5] lb	130.5	137.3	138.7	2.51	0.04	0.02	0.36
Weight change, lb							
Gestation	14.3	19.6	20.1	1.30	0.01	0.01	0.15
Lambing	-27.7	-24.8	-26.4	1.30	0.33	0.50	0.16
Percent BW change,[6] %	-10.65	-4.98	-4.98	1.02	<0.001	<0.001	0.02
Initial BCS	2.9	2.9	2.9	0.03	0.51	0.53	0.33
BCS at lambing[5]	2.7	2.7	2.9	0.06	0.02	0.01	0.45
BCS change							
Gestation	-0.02	0.02	0.02	0.04	0.68	0.44	0.65
Lambing							
d 100 to lambing[7]	-0.20	-0.17	0.02	0.05	0.02	0.01	0.24
d 142 to lambing[8]	-0.18	-0.20	-0.02	0.05	0.06	0.05	0.14
Lamb birth weight, lb	10.0	9.9	10.4	0.24	0.30	0.22	0.33
Lamb weaning BW, lb	38.7	43.0	42.2	1.46	0.07	0.08	0.14
Lamb ADG,[9] lb/d	0.40	0.46	0.44	0.01	0.07	0.10	0.10

[1]Maternal diets (DM basis) were balanced for mature ewes baring twins during the last 4 weeks of gestation according to NRC (2007). Treatments: 60MP1: 60% of metabolizable protein of 100MP1; 80MP1: 80% of the metabolizable protein 100MP1; and 100MP1: 100% of the metabolizable protein requirement.
[2]Greatest SEM presented (n = 7).
[3]*P* -value for the F test of the mean.
[4]*P*-value for linear and quadratic effects of increasing metabolizable protein concentrations.
[5]Ewe BW and BCS measured within 24 h after parturition.
[6]Percent BW change from initial BW (d 100 of gestation) to BW immediately postpartum.
[7]Change in ewe BCS from the initial BCS (d 100 of gestation) to the BCS immediately postpartum.
[8]Change in ewe BCS from the final BCS (d 142 of gestation) to the BCS immediately postpartum.
[9]ADG calculated: (weight at birth, kg – weight at weaning)/age at weaning.

Table 4. Effects of maternal metabolizable protein supplementation during the last 50 d of gestation on ewe performance and offspring performance from birth to weaning for year 2

	Dietary Treatment[1]					Orthogonal Contrasts[4]	
Item	60MP2	100MP2	140MP2	SEM[2]	*P* – value[3]	Linear	Quadratic
Initial BW, lb	148.7	148.6	148.4	3.55	1.00	0.95	0.97
BW at lambing,[5] lb	134.0	142.0	144.1	3.17	0.06	0.02	0.48
Weight change, lb							
Gestation	11.4	17.6	19.2	1.72	0.02	0.01	0.28
Lambing	-27.8	-25.0	-24.8	1.90	0.35	0.26	0.60
Percent BW change,[6] %	-11.62	-5.09	-4.04	1.44	<0.001	<0.001	0.12
Initial BCS	3.0	3.0	3.0	0.01	0.40	0.22	0.40
BCS at lambing[5]	2.9	3.0	3.0	0.04	0.93	0.74	0.88
BCS change							
Gestation	0.00	0.00	-0.02	0.01	0.40	0.22	0.49
Lambing							
d 100 to lambing[7]	-0.06	-0.04	-0.04	0.04	0.94	0.74	0.88
d 142 to lambing[8]	-0.06	-0.04	-0.02	0.04	0.81	0.51	0.96
Lamb birth weight, lb	10.2	10.6	10.2	0.24	0.45	0.92	0.22
Lamb weaning BW, lb	33.6	36.8	35.4	1.87	0.49	0.51	0.33
Lamb ADG,[9] lb/d	0.40	0.44	0.40	0.02	0.25	0.78	0.11

[1]Maternal diets (DM basis) were balanced for mature ewes baring twins during the last 4 weeks of gestation according to NRC (2007). Treatments: 60MP2: 60% of metabolizable protein of 100MP2; 140MP1: 140% of the metabolizable protein 100MP2; and 100MP2: 100% of the metabolizable protein requirement.
[2]Greatest SEM presented (n = 4).
[3]*P* -value for the F test of the mean.
[4]*P*-value for linear and quadratic effects of increasing metabolizable protein concentrations.
[5]Ewe BW and BCS measured within 24 h after parturition.
[6]Percent BW change from initial BW (d 100 of gestation) to BW immediately postpartum.
[7]Change in ewe BCS from the initial BCS (d 100 of gestation) to the BCS immediately postpartum.
[8]Change in ewe BCS from the final BCS (d 142 of gestation) to the BCS immediately postpartum.
[9]ADG calculated: (weight at birth, kg – weight at weaning)/age at weaning.

Effects of maternal metabolizable protein supplementation during the last 50 days of gestation on male and female offspring performance post-weaning[1]

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The objectives of this trial were to determine the effects of maternal metabolizable protein supplementation in ewes during the last 50 days of gestation on male and female offspring performance post-weaning. Results suggest restricting MP intake to 60% of requirements to ewes during late gestation may negatively impact F1 offspring growth and reproductive performance, beginning with F1 birth weights.

INTRODUCTION

Crude protein supplementation not only allows dams to maintain BW and BCS, but appears to improve offspring performance (Stalker et al., 2006; Larson et al., 2009). Crude protein supplementation to the dam is just one method of improving livestock performance during gestation. Metabolizable protein has been defined as the protein and amino acids that are digested and absorbed post-ruminally (Burroughs et al., 1975). Since MP is the protein directly available to the dam, it may be an indicator of how protein intake during gestation will ultimately affect offspring performance both pre- and post-weaning. However, there has been minimal research conducted on the effects of MP intake during late gestation in sheep on offspring performance.

We hypothesized that the greater proportion of the diet that is composed of MP would yield improved offspring growth by potentially increasing nutrient transfer by the placenta or by increased nutrients within the milk. Therefore, the objectives were to evaluate isocaloric diets with increasing levels of MP during late gestation on male and female offspring performance post-weaning.

PROCEDURES

All procedures were approved by the NDSU Animal Care and Use Committee. This study was conducted at the Hettinger Research Extension Center in Hettinger, ND.

Ewes. On d 100 ± 8 (SD) of gestation, using the average of the initial weights (d 99 and 100 of gestation), ewes were stratified by BW, BCS, age, and expected lambing date to one of three isocaloric dietary treatments (Table 2; n = 7): **100MP1**: 100% of the MP requirements on a DM basis during the last 4 weeks of gestation of a ewe carrying twins (NRC, 2007); **60MP1**: 60% of 100MP1; and **80MP1**: 80% of 100MP. Isocaloric dietary treatments (Table 2; n = 4) in year 2 were: **60MP2**: 60% of MP requirements; **100MP2**: 100% of the MP requirements; and **140MP2**: 140% of MP requirements on a DM basis of a ewe carrying twins during the last 4 weeks gestation (NRC, 2007). Once ewes had lambed, the ewes and lambs were intermingled between dietary treatments where they were maintained on a lactation ration (Table 1).

Lambs. In both years, lambs were weighed and tagged within

[1]This project was supported by National Research Initiative Competitive Grant no. 2009-35206-05276 from the USDA National Institute of Food and Agriculture. The authors would like to thank David Pearson, Donald Drolc, Donald Stecher, Tammi Neville, and James Kirsch for their assistance in conducting this trial.

24 h of birth; sex, lambing assistance, and lamb vigor were also recorded. Lambs were then moved with the ewes to grouping pens and had full-access to creep pellet and water prior to weaning. At 14 days of age and at weaning all lambs were vaccinated for tetanus and *Clostridium Perfringens* types C and D (CD-T; Bar Vac CD-T, Boehringer Ingelhein, Ridgefield, CT), tails were docked, and ram lambs were castrated. Lambs were weaned at 69 ± 5 d (SD) of age in year 1 and at 61 ± 12 (SD) d of age in year 2 and weighed.

Feedlot. In year 1, at 89 ± 5 (SD) days of age and in year 2, at 102 ± 11 (SD) days of age, wether lambs were revaccinated for CD-T and placed in the feedlot. In both years, wethers were allotted by maternal dietary treatment and blocked by weight (heavy and light) into one of two pens per maternal dietary treatment. Wethers were fed approximately 85% whole, shelled corn and 15% commercial market lamb pellet diet (Table 3). The feedlot ration was balanced to meet or exceed CP and NE requirements of growing lambs (NRC, 2007). Wethers were fed a mixed diet ad libitum via bulk feeders. Lambs had continuous access to fresh water and shade. In year 1, wether lambs were shipped to the Iowa Lamb Corporation (Hawarden, IA) or the NDSU Meat Lab (Fargo, ND) for carcass measurements. In year 2, all wethers were shipped to Superior Farms (Denver, CO).

Ewe lambs. In both years, F1 lambs were fed a mixed diet ad libitum via bulk feeders (Table 3). In year 1, between 108 ± 10 (SD) and 236 ± 10 (SD) days of age,

Table 1. Nutrient composition of fescue straw and lactation ration in years 1 and 2

	Fescue Straw[1]		
Item	Year 1	Year 2	Lactation Ration[2]
Diet, % DM	64.51	56.51	100.00
DM, %	83.05	77.61	64.37
NEm, Mcal/kg	2.22	2.12	—
CP, % of DM	3.04	3.07	11.52
MP, % of DM	1.95	1.97	—
NDF, % of DM	79.85	81.13	48.05
ADF, % of DM	48.97	51.10	27.16
Ash, % of DM	9.49	7.78	8.59

[1]Ewes were fed fescue straw in each year to limit metabolizable protein intake.

[2]Ewes were fed a common ration during lactation across all dietary treatments; 28.5% oats, 28.5% haylage, 42.9% chopped hay.

Table 2. Ingredient and nutrient composition of dietary supplements fed to ewes in year 1 and 2

	Year 1[1]			Year 2[2]		
Item	60MP1	80MP1	100MP1	60MP2	100MP2	140MP2
Ingredient, % DM						
Corn	18.50	15.00	5.00	30.00	19.00	—
DDGS[3]	7.00	20.00	30.00	4.00	24.00	43.00
Soyhulls	9.50	—	—	9.00	—	—
Trace mineral[4]	0.49	0.49	0.49	0.49	0.49	0.49
Nutrient composition						
DM, %	88.75	89.34	89.68	88.64	90.19	92.16
NEm, Mcal/kg	2.00	2.22	2.14	2.05	2.19	2.06
CP, % of DM	13.16	20.21	25.13	10.21	18.67	28.68
MP, % of DM	8.41	13.01	16.31	6.54	11.96	18.37
NDF, % of DM	31.03	30.73	39.79	29.64	31.40	45.34
ADF, % of DM	15.69	7.45	10.49	13.87	8.68	13.34
Ash, % of DM	3.22	3.48	4.55	3.53	3.80	5.13

[1]Maternal diets (DM basis) were balanced for mature ewes baring twins during the last 4 weeks of gestation according to NRC (2007). Treatments: 60MP1: 60% of metabolizable protein of 100MP1; 80MP1: 80% of the metabolizable protein 100MP1; and 100MP1: 100% of the metabolizable protein requirement.

[2]Maternal diets (DM basis) were balanced for mature ewes baring twins during the last 4 weeks of gestation according to NRC (2007). Treatments: 60MP2: 60% of metabolizable protein of 100MP2; 140MP2: 140% of the metabolizable protein of 100MP2; 100MP2: 100% of the metabolizable protein requirement.

[3]Dried distillers grains with solubles

[4]Trace mineral content: 16.0-17.0% Ca; 8.0% P; 21.0-23.0% Salt; 2.75% Mg; 3 ppm Co; 5 ppm Cu; 100 ppm I; 1,400 ppm Mn; 20 ppm Se; 3,000 ppm Zn; 113,500 IU/kg Vitamin A; 11,350 IU/kg Vitamin D; and 227 IU/kg Vitamin E.

F1 growth performance was measured. Body weights were taken every 14 days throughout the 128 day period. Growth performance in year 2 was measured from 63 ± 13 (SD) day of age to 191 ± 20 (SD) days of age. Body weights were taken at the beginning (d 0) and at the end (d 128) of the 128 day period. F1 breeding began at 259 ± 10 (SD) days of age in year 1 and 256 ± 9 (SD) days of age in year 2. F1 were maintained in a single flock during the 51 day breeding period. Rambouillet rams (n = 10) were fitted with marking harnesses and were introduced to the flock. Breeding harness crayons were changed to a different color on d 18 and 35 days post-ram introduction. Rams were removed from the pen 51 days after the rams were introduced. In both years, pregnancy was confirmed via ultrasonography 45 days after the rams were removed. In both years, lambs of F1 dams (**F2**) were treated similarly as lambs from ewes discussed previously.

Statistical analysis. Feedlot performance, carcass characteristics, F1 performance, and F1 reproductive efficiency were analyzed utilizing the MIXED procedures of SAS (SAS Inst. Inc., Cary, NC). When a significant *F*-test was observed ($P \leq 0.15$), pre-planned comparisons of linear and quadratic contrasts were utilized to partition treatment effects. Significance was set at $P \leq 0.05$ and tendencies at $P \leq 0.10$.

Year 1

Wethers. There was no effect ($P \geq 0.33$; Table 4) of maternal dietary treatment on initial BW, final BW, ADG, G:F, or morbidity. There was a quadratic effect ($P = 0.04$) for days on feed, with the 80MP1 lambs being on feed longer than the 60MP1 and 100MP1 lambs. A quadratic effect ($P = 0.05$) was also observed for DMI, with the 80MP1 wethers consuming less feed than the 60MP1 and 100MP1 wethers. There was no effect ($P \geq 0.27$; Table 4) of maternal MP intake during late gestation on carcass measurements, except a linear tendency ($P = 0.06$) for flank streaking to increase in wethers as MP increased in the late gestation ewe diet.

Table 3. Ingredient and nutrient composition of diets fed to feedlot wether lambs and ewe lambs in years 1 and 2

	Wethers		Ewe Lambs
Item	Year 1	Year 2	
Ingredient, %			
Oats	—	—	60.0
Whole corn	84.4	84.7	20.0
Market lamb pellet[1]	15.6	15.3	20.0
Nutrient composition			
DM, %	89.06	89.54	91.1
CP, % of DM	13.12	13.50	22.3
NDF, % of DM	13.48	22.93	23.8
ADF, % of DM	3.42	4.23	10.1
Ash, % of DM	4.59	5.52	10.5

[1]Commercial Market Lamb Pellet contained: 0.22 g/kg Chlortetracycline; 38.0% CP; 3.75-4.75% Ca; 0.6% P; 3.0-4.0% salt; 1.2 ppm Se; 52,863 IU/kg Vitamin A; 5,286 IU/kg Vitamin D; and 209 IU/kg Vitamin E.

Ewe lambs. Weaning BW and ADG from birth to weaning, weaning to the end of the 128 d growth period, birth to the end of the 128 d growth period, initial BW, final BW, and ADG during the 128 day growth period were not altered ($P \geq 0.22$; Table 5) due to maternal MP dietary treatment. There was a quadratic effect ($P = 0.003$) for F1 birth weight, with the F1 offspring from 80MP1 ewes having increased birth weights compared with F1 from 60MP1 and 100MP1 ewes. The total percentage of F1 lambing, F1 lambing in the third 17 days of the lambing period, lambing rate, or on birth weight of F2 was not different ($P \geq 0.22$; Table 5) due to maternal dietary treatment. However, a quadratic effect ($P = 0.02$) was observed for the percentage of F1 being marked for breeding during the first 17 day breeding cycle with F1 born to ewes fed 80MP1 being increased compared with F1 born to ewes fed 60MP1 and 100MP1. F1 lambing to the first 17 day breeding cycle was increased linearly ($P = 0.001$) as MP intake increased in the maternal diet during late gestation. The percentage of F1 lambing during the second 17 day breeding cycle decreased linearly ($P = 0.02$) as MP intake in the maternal diet increased during late gestation.

Year 2

Wethers. In year 2, there was no effect ($P \geq 0.12$; Table 6) of maternal dietary treatment on initial BW, final BW, ADG, G:F, or morbidity. There was a linear increase ($P = 0.04$) in DMI as

maternal MP increased from 60 to 140% of MP requirements. Carcass characteristics were not affected ($P \geq 0.40$; Table 6) by ewe MP intake during late gestation.

Ewe lambs. Average daily gain during the 128 day growth period, from birth to the end of the 128 day growth period, and F1 birth weights were not altered ($P \geq 0.17$; Table 7) by maternal MP treatment. However, there tended ($P = 0.09$) to be a quadratic effect on weaning weights, where F1 from 100MP2 ewes tended to be increased compared with F1 from 60MP2 and 140MP2 ewes. There was a quadratic effect ($P = 0.01$) for ADG from birth to weaning of F1 from ewes consuming 100% of MP requirements being increased compared with F1 from ewes fed 60 and 140% of MP requirements. There tended to be a quadratic effect ($P = 0.07$) for final BW at the end of the growth period, where F1 from 100MP2 ewes weighed more on d 128 of the growth period compared with F1 from 60MP2 and 140MP2 ewes. There was no effect ($P \geq 0.76$; Table 7) of maternal MP treatment on the total percentage of F1 lambing or the percentage of F1 lambing to the first, second

Table 4. Effects of maternal metabolizable protein supplementation on feedlot performance and carcass characteristics of wethers in year 1

	Maternal Dietary Treatment[1]					Orthogonal Contrasts[4]	
Item	60MP1	80MP1	100MP1	SEM[2]	*P* – value[3]	Linear	Quadratic
Feedlot							
Initial BW, lb	64.4	62.2	65.7	4.59	0.85	0.82	0.60
Final BW, lb	153.9	150.8	146.6	3.51	0.33	0.14	0.89
Days on feed, d	127	133	123	3.8	0.10	0.38	0.04
ADG, lb/d	0.71	0.68	0.66	0.02	0.40	0.18	0.93
DMI, lb/lamb/d	3.31	3.17	3.28	0.04	0.13	0.84	0.05
G:F, lb gain:lb DMI	0.23	0.22	0.21	0.01	0.64	0.36	0.79
Morbidity,[5] %	6.4	20.5	20.2	9.1	0.33	0.22	0.43
Carcass characteristics							
HCW, lb	79.6	77.2	75.6	2.01	0.33	0.14	0.88
Dressing Percentage, %	51.8	51.2	51.4	0.44	0.53	0.53	0.38
LM area, in^2	2.74	2.74	2.73	0.08	0.95	0.76	0.94
Back fat thickness, in	0.28	0.31	0.28	0.02	0.27	0.43	0.14
Body wall thickness, in	1.10	1.06	1.06	0.04	0.72	0.42	0.92
Leg score[6]	12	12	12	0.2	0.84	0.61	0.74
Conformation score[6]	12	12	12	0.2	0.64	0.98	0.35
Flank streaking[7]	362	365	395	13.5	0.13	0.06	0.36
Quality grade[6]	12	12	12	0.1	0.38	0.17	0.98
Yield grade[8]	3.3	3.5	3.2	0.3	0.54	0.85	0.27
BCTRC,[9] %	44.79	44.94	48.56	2.01	0.34	0.19	0.48
WBSF,[10] lb	7.23	6.00	6.19	0.68	0.40	0.30	0.40

[1]Maternal dietary treatment: 60MP1: 60% of metabolizable protein of 100MP1; 80MP1: 80% of the metabolizable protein 100MP1; and 100MP1: 100% of the metabolizable protein requirement.
[2]Greatest SEM presented (n = 31 for 60MP1, n = 33 for 80MP1, and n = 24 for 100MP1).
[3]*P*-value for the F test of the mean.
[4]*P*-value for linear and quadratic effects of increasing metabolizable protein concentrations.
[5]Percentage treated for illness during the feedlot phase.
[6]Leg score, conformation score, and quality grade: 1 = cull to 15 = high prime.
[7]Flank streaking: 100-199 = practically devoid; 200-299 = traces; 300-399 = slight; 400-499 = small; 500-599 = modest.
[8]Yield grade = (back fat thickness × 10) + 0.4.
[9]Percent boneless, closely trimmed, retail cuts (% BCTRC) = [49.936 – (0.0848 × HCW, lb) – (4.376 × back fat thickness, in) – (3.53 × body wall thickness, in) + (2.456 × LM area, in^2)].
[10]Warner-Bratzler shear force.

17 days of the lambing season, and lambing rate. There tended to be a quadratic effect ($P = 0.09$) for F1 from ewes fed 100MP2 having increased F1 breeding during the first 17 days of breeding compared with F1 from 60MP2 and 140MP2 ewes. F1 from 60MP2 and 100MP2 fed ewes gave birth to heavier ($P = 0.05$) lambs than F1 from 140MP2 fed ewes. Birth weights of F2 born to F1 were reduced linearly ($P = 0.04$) as grand-dam MP intake increased during late gestation.

DISCUSSION

Performance during the feedlot phase in the current study was not affected by maternal MP intake. Previously, Larson et al. (2009) observed no differences in steer ADG, DMI, or G:F due to maternal dietary CP supplementation. Additionally, Stalker et al. (2006) reported no differences in calf ADG, DMI, or G:F during the finishing phase due to maternal CP supplementation. Overall, the current results suggest that feedlot performance in sheep may not be altered by maternal MP intake during late gestation.

Similar to the current results, Stalker et al. (2006) did not observe any differences in steer

Table 5. Effects of maternal metabolizable protein supplementation on ewe lamb growth and reproductive performance in year 1

	Maternal Dietary Treatment[1]					Orthogonal Contrasts[4]	
Item	60MP1	80MP1	100MP1	SEM[2]	*P* – value[3]	Linear	Quadratic
Birth weight, lb	9.9	11.5	10.8	0.31	0.002	0.05	0.003
Weaning BW, lb	40.1	43.9	42.3	2.16	0.41	0.41	0.28
ADG, lb/d							
Birth to weaning	0.42	0.49	0.46	0.029	0.37	0.36	0.26
Weaning to final[5]	0.60	0.60	0.60	0.015	0.78	0.55	0.74
Birth to final[5]	0.55	0.57	0.55	0.013	0.43	0.86	0.20
Growth period[6]							
Initial BW, lb	68.6	73.9	67.2	3.31	0.29	0.22	0.13
Final BW, lb	139.6	146.9	139.1	3.68	0.22	0.16	0.08
ADG, lb/d	0.55	0.57	0.57	0.018	0.80	0.61	0.55
Breeding in first 17 days,[7] %	50	84	67	9.3	0.03	0.15	0.02
Total lambing,[8] %	70	68	67	9.5	0.96	0.78	0.98
Lambing to first 17 days,[9] %	0	0	32	7.3	0.001	0.001	0.07
Lambing to second 17 days,[9] %	86	65	52	11.2	0.05	0.02	0.75
Lambing to third 17 days,[9] %	14	35	16	9.8	0.22	0.89	0.08
Lambing rate,[10]	0.73	0.80	0.81	0.12	0.88	0.64	0.83
Lamb birth weight, lb	10.6	10.6	10.4	0.44	0.85	0.64	0.78

[1]Maternal dietary treatment: 60MP1: 60% of metabolizable protein requirements; 80MP1: 80% of metabolizable protein requirements; and 100MP1: 100% of metabolizable protein requirements.

[2]Greatest SEM presented (n = 30 for 60MP1, n = 25 for 80MP1, and n = 36 for 100MP1).

[3]*P*-value for the F test of the mean.

[4]*P*-value for linear and quadratic effects of increasing metabolizable protein concentrations.

[5]Weaning to final indicates the ADG from weaning to the final BW measured on d 128 of the 128 day growth period. Birth to final indicates the ADG from birth to the final BW measured on d 128 of the 128 day growth period.

[6]Growth period that was 128 days in length to measure growth performance of the ewe lambs.

[7]Percentage of ewe lambs per treatment having breeding marks in the first 17 days of the breeding season.

[8]Total percentage of ewe lambs lambing per ewe lamb exposed per maternal dietary treatment.

[9]Percentage of ewe lambs lambing that were bred during the first 17 days post-ram turnout, the second 17 days post-ram turnout, or the third 17 days post-ram turnout.

[10]Lambing rate: number of lambs born per ewe exposed to the rams.

carcass measurements due to maternal CP supplementation during late gestation on steer offspring. However, similar to year 1, Larson et al. (2009) observed increased marbling scores steers born to cows that were supplemented with CP during late gestation. The current results suggest that maternal MP intake in isocaloric diets may have little impact on carcass characteristics of wether offspring.

To our knowledge, there has been little research conducted evaluating the feeding of MP or CP during late gestation in ruminants on their effects of female offspring post-weaning. Martin et al. (2007) observed an increase in adjusted 205 d BW, prebreeding BW, and BW at pregnancy diagnosis in heifers born to cows that were supplemented with CP during late gestation. As our results suggest, restricting MP intake to 60% of requirements to ewes during late gestation may negatively impact F1 offspring growth and reproductive performance, beginning with F1 birth weights.

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Table 6. Effects of maternal metabolizable protein supplementation on feedlot performance and carcass characteristics of wethers in year 2

	Maternal Dietary Treatment[1]					Orthogonal Contrasts[3]	
Item	60MP2	100MP2	140MP2	SEM[2]	*P* – value[3]	Linear	Quadratic
Feedlot							
Initial BW, lb	55.8	62.4	65.0	4.59	0.36	0.18	0.71
Final BW, lb	131.6	138.2	139.4	4.87	0.48	0.27	0.65
ADG, lb/d	0.53	0.55	0.53	0.02	0.82	0.79	0.59
DMI, lb/lamb/d	2.25	2.31	2.47	0.07	0.09	0.04	0.66
G:F, lb gain:lb DMI	0.24	0.24	0.22	0.02	0.60	0.35	0.73
Morbidity,[4] %	13.6	31.8	9.1	10.0	0.12	0.69	0.04
Carcass characteristics							
HCW, lb	71.9	71.2	71.2	2.84	0.99	0.89	0.92
Dressing percentage, %	49.9	49.4	49.3	0.46	0.62	0.38	0.65
LM area, in^2	2.56	2.54	2.59	0.09	0.94	0.83	0.81
Back fat thickness, in	0.28	0.28	0.24	0.02	0.56	0.30	0.96
Body wall thickness, in	1.10	0.91	0.98	0.06	0.40	0.41	0.29
Leg score[5]	12	12	12	0.3	0.55	0.54	0.39
Conformation score[5]	12	12	12	0.3	0.86	0.59	0.93
Flank streaking[6]	396	409	398	18.2	0.83	0.96	0.54
Quality grade[5]	12	12	12	0.3	0.78	0.97	0.48
Yield grade[7]	3.2	3.0	2.9	0.25	0.56	0.30	0.96
BCTRC,[8] %	45.16	45.69	45.69	0.38	0.50	0.30	0.53

[1]Maternal dietary treatment: 60MP2: 60% of metabolizable protein of 100MP2; 100MP2: 100% of the metabolizable protein requirement; and 140MP2: 140% of the metabolizable protein requirement.
[2]Greatest SEM presented (n = 20 for 60MP2, n = 22 for 100MP2, and n = 20 for 140MP2).
[3]*P*-value for the F test of the mean.
[4]*P*-value for linear and quadratic effects of increasing metabolizable protein concentrations.
[5]Percentage treated for illness during the feedlot phase.
[5]Leg score, conformation score, and quality grade: 1 = cull to 15 = high prime.

[6]Flank streaking: 100-199 = practically devoid; 200-299 = traces; 300-399 = slight; 400-499 = small; 500-599 = modest.
[7]Yield grade = (back fat thickness × 10) + 0.4.
[8]Percent boneless, closely trimmed, retail cuts (% BCTRC) = [49.936 – (0.0848 × HCW, in) – (4.376 × back fat thickness, in) – (3.53 × body wall thickness, in) + (2.456 × LM area, in^2)].

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Table 7. Effects of maternal metabolizable protein supplementation on ewe lamb growth and reproductive performance in year 2

	Maternal Dietary Treatment[1]					Orthogonal Contrasts[4]	
Item	60MP2	100MP2	140MP2	SEM[2]	*P* – value[3]	Linear	Quadratic
Birth weight, lb	9.9	9.9	10.4	0.46	0.62	0.43	0.52
Weaning BW, lb	32.4	37.3	35.3	1.61	0.07	0.14	0.09
Final BW,[5] lb	131.2	139.1	126.1	4.76	0.14	0.37	0.07
ADG, lb/d							
Birth to weaning	0.40	0.44	0.37	0.02	0.03	0.25	0.01
Weaning to final[6]	0.51	0.55	0.51	0.02	0.48	0.79	0.23
Birth to final[6]	0.49	0.51	0.46	0.02	0.17	0.55	0.07
Breeding in first 17 days,[5] %	84	94	70	9.0	0.12	0.18	0.09
Total lambing,[6] %	19	28	26	10.2	0.76	0.57	0.65
Lambing to first 17 days,[7] %	86	88	83	15.4	0.98	0.91	0.86
Lambing to second 17 days,[7] %	14	13	17	15.4	0.98	0.91	0.86
Lambing rate[8]	0.23	0.33	0.26	0.12	0.78	0.80	0.52
Lamb birth weight, lb	10.4	10.4	7.9	0.86	0.05	0.04	0.14

[1]Maternal dietary treatment: 60MP2: 60% of metabolizable protein requirements; 100MP2: 100% of metabolizable protein requirements; and 140MP2: 140% of metabolizable protein requirements.

[2]Greatest SEM presented (n = 31 for 60MP2, n = 18 for 100MP2, and n = 23 for 140MP2).

[3]*P*-value for the F test of the mean.

[4]*P*-value for linear and quadratic effects of increasing metabolizable protein concentrations.

[5]Final BW observed at the end of the 128 day growth period beginning at weaning.

[6]Weaning to final indicates the ADG from weaning to the final BW measured on d 128 of the 128 day growth period. Birth to final indicates the ADG from birth to the final BW measured on d 128 of the 128 day growth period.

[5]Percentage of ewe lambs per treatment having breeding marks in the first 17 days of the breeding season.

[6]Total percentage of ewe lambs lambing per ewe lamb exposed per maternal dietary treatment.

[7]Percentage of ewe lambs lambing that were bred during the first 17 days post-ram turnout and the second 17 days post-ram turnout.

[8]Lambing rate: number of lambs born per ewe exposed to rams.

Effects of rumen-protected arginine supplementation during gestation in ewes on postnatal offspring performance[1]

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Maternal under nutrition can have serious negative effects on fetal development and postnatal outcomes, and is relatively common in grazing ewes (Wu et al., 2006). For this reason, we supplemented pregnant multiparous ewes that were restricted in nutrition with a rumen-protected arginine supplement in an attempt to mitigate negative consequences of compromised maternal nutrition during gestation. We found that lambs from restricted ewes had reduced birth weights and that providing a rumen-protected arginine supplement to ewes during gestation recovered lamb body weight by 19 days of age. Additional research is needed to determine if arginine supplementation could be a means for producers to enhance postnatal lamb performance.

INTRODUCTION

Death losses in the United States sheep industry are notably higher in lambs than adult sheep. Most lamb death losses occur before reaching the age of being marked, docked, or branded (USDA, 2012). Neonatal lamb death is an economic issue and methods to enhance lamb growth, development, and health from a young age with a focus on reducing lamb morbidities and mortalities while simultaneously improving performance should yield benefits for producers.

Studies have shown that the body weights and gain potential of lambs may be heavily influenced by maternal nutrition (Wu et al., 2006, Meyer et al., 2010, Neville et al., 2010). Because most ewes maintain their pregnancies throughout late fall and winter, availability of high quality forages may be compromised and maternal under nutrition can result. In fact, it is estimated that grazing ewes in the western U.S. often don't meet even 50% of National Research Council (NRC) recommendations; clearly supplementation is critical in these instances (Wu et al., 2006). If maternal under nutrition occurs throughout pregnancy, fetal growth and postnatal outcomes can be compromised. Developing offspring from undernourished ewes often are at higher risk of several animal health complications including respiratory diseases, which are implicated as one of the highest non-predator causes of lamb death loss in the U.S. (Wu et al., 2006, USDA, 2012).

Arginine, an amino acid, is a potential supplement that may help to circumvent under nutrition of grazing ewes. Arginine, among numerous other functions, serves as a precursor to nitric oxide and polyamines (Wu and Morris, 1998, Kwon, 2003). Nitric oxide is a known vasodilator which serves to increase blood flow to the fetus, and in turn transports more nutrients to the placenta for fetal development (Martin et al., 2001). Polyamines play various roles in placental health and development, most notably in regulation of angiogenesis, or the formation of new blood vessels. Similarly to nitric oxide, these polyamines may stimulate blood flow and consequently increase nutrient supply to the placenta throughout gestation (Kwon, 2003). We hypothesize that ewes supplemented with arginine

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throughout pregnancy will have a greater amount of nutrients available to the fetus during development, and their lambs will show more advanced development evidenced by increased weight gain.

PROCEDURES

Ewes. Thirty-two multiparous western white-face ewes were obtained from Hettinger Research Extension Center in Hettinger, North Dakota. The ewes were confirmed pregnant via ultrasound, and randomly assigned to three treatments: control (**CON**), restricted (**RES**), and restricted with an arginine supplement (**RES-ARG**). Ewes were fed a pelleted diet containing 34% dehydrated alfalfa meal, 27% dehydrated beet pulp, 25% wheat middlings, 9% ground corn, 5% soybean meal, and a tracemineral premix exchanged for ground corn at the rate of 12 pounds per ton on an as fed basis. Control ewes were fed at 100% NRC requirements, while restricted ewes were fed 60% of NRC requirements, and the arginine supplemented group received a granular rumen-protected arginine supplement at 180 mg/kg BW daily. Rumen protected arginine supplements were mixed into 0.11 pounds of corn and fed once a day at 8:00 am. Dietary treatments were implemented at day 54 of pregnancy (standard deviation of start date was 3.89 days). Ewes were housed in individual pens in a temperature-controlled facility.

Lambing. A 24-hour ewe watch procedure was implemented during lambing. Lambs were tagged, weighed, and a blood sample collected immediately following birth. They received C, D, & Tetanus toxoid and vitamins A, D, and E injections post birth. These lambs were not permitted to nurse from their mothers, so artificial colostrum was administered according to requirements of the lamb indicated by weight (Lifeline Rescue Colostrum, APC, Ankeny, IA). Lambs were given 19.1 mL/kg BW colostrum at intervals of 0 and 2 hours post birth, and 25.5 mL/kg BW at intervals of 4, 8, 12, 16, and 20 hours post birth to achieve a total of 10.64g IgG/kg body weight.

Lambs. After 24 hours, lambs were gradually weaned off of bottles to teat buckets filled with milk replacer (Super Lamb Milk Replacer, Merrick's Inc., Middleton, WI). This milk replacer, along with water, was available to them ad libitum. In addition to the milk replacer, a mixture of alfalfa hay and creep feed (Form-A-Feed 20% Lamb Pre-Starter, Form-A-Feed Inc. Stewart, MN) was also available ad libitum. Curved crown rump and girth measurements were taken post birth, and at 19 and 54 days of age. Lambs were weighed at birth, 24 hours, 3, 7, 14 ± 3, 19 ± 3, 33 ± 3, 40 ± 3, 47 ± 3, and 54 ± 3 days of age. Weighing procedures and scales remained constant throughout the project.

RESULTS AND DISCUSSION

Body weights of lambs from CON ewes were greater ($P < 0.05$) than lambs from RES ewes at days 0 ($P = 0.04$), 3 ($P = 0.003$), 7 ($P = 0.03$), 14 ($P = 0.02$), 19 ($P = 0.004$), and 33 ($P = 0.012$). Lambs from RES and RES-ARG ewes had similar body weights at birth ($P = 0.68$), weighing less than lambs from CON ewes (Figure 1). Lambs from RES-ARG ewes tended to weigh less than lambs from CON ewes at birth and on day 7 ($P = 0.10$, $P = 0.08$, respectively), and weighed significantly less on day 3 ($P = 0.02$). However, by day 19 lambs from RES-ARG ewes weighed more than lambs from RES ewes ($P = 0.04$), and were more similar to weights of lambs from CON ewes ($P = 0.41$). At day 19, lambs from CON ewes weighed 26.40 pounds, lambs from RES ewes weighed 22.65 pounds, and lambs from RES-ARG ewes weighed 25.35 pounds (Figure 1). Although birth weights of lambs from RES and RES-ARG ewes were similar, the lambs from RES-ARG ewes caught up to the lambs from CON ewes over time (Figure 1).

Average daily gains are shown in Fig. 2. Compared to lambs from RES ewes, lambs from RES-ARG ewes had greater ADG on day 19 ($P = 0.04$) and numerically had higher ADG for each time period during this trial. At day 19, lambs from CON ewes were gaining 0.783 pounds per day, lambs from RES ewes were gaining 0.676 pounds per day, and lambs from RES-ARG ewes were gaining 0.780 pounds per day (Figure 2).

Table 1 shows differences in curved crown rump and girth measurements. Lambs from RES-ARG ewes were not different than lambs from CON ewes in girth ($P > 0.05$), and, were different from lambs from RES ewes on day 19 ($P = 0.02$) . Girth measurements on day 19 showed lambs from CON ewes were 21.81 inches, lambs from RES ewes were 20.20 inches, and lambs from RES-ARG ewes were 21.50 inches (Table 1). The only difference observed for curved

crown rump measurements was on day 54; lambs from RES-ARG ewes had greater curved crown rump than lambs from RES ewes ($P = 0.003$). Curved crown rump measurements on day 54 were 37.91 inches for lambs from CON ewes, 36.97 inches for lambs from RES ewes, and 39.31 inches for lambs from RES-ARG ewes (Table 1).

IMPLICATIONS

These results imply that supplementing ewes with arginine during pregnancy may circumvent the effects of under nutrition. By avoiding these deleterious consequences of poor weight gains, producers could expect to have more vigorous lambs and lower lamb mortality rates. This would ultimately translate in to higher profitability for producers.

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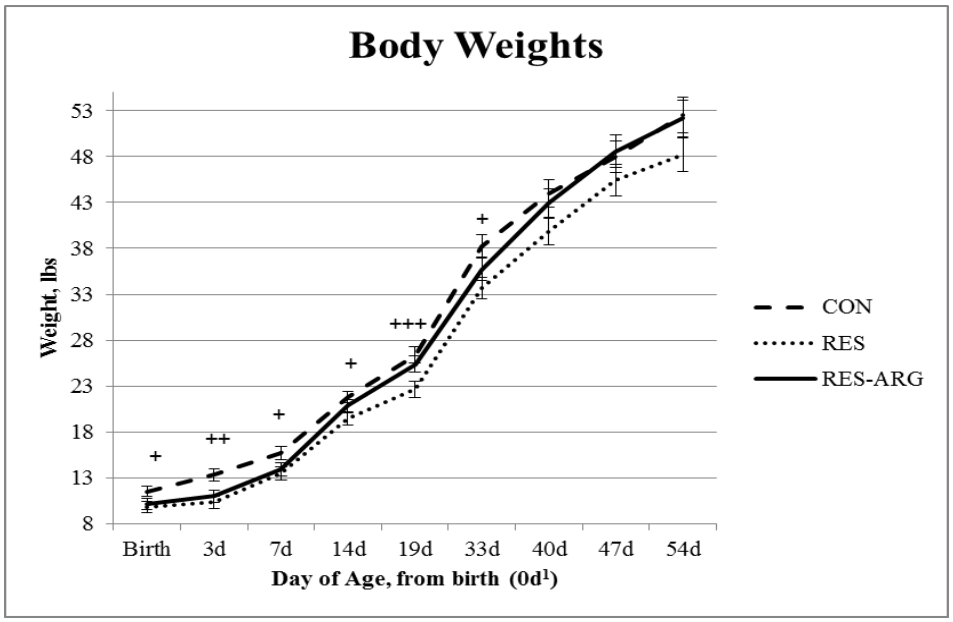
Table 1. Influence of nutrient restriction and rumen-protected arginine supplementation to ewes on offspring girth and curved crown rump measurements over time

Item	CON	RES	RES-ARG	SEM	*P*-value
Girth (in.)					
Birth (0d[1])	16.61[b]	15.19[a]	15.50[ab]	0.449	0.08
19d	21.81[b]	20.20[a]	21.50[b]	0.384	0.01
54d	27.89[b]	26.38[a]	27.43[ab]	0.486	0.10
CCR[c] (in.)					
Birth (0d[1])	21.62	20.71	21.69	0.585	0.43
19d	29.03	27.33	28.70	0.731	0.21
54d	37.91[ab]	36.97[a]	39.31[b]	0.502	0.01

[a,b] Means within a row with different superscripts differ ($P < 0.05$).
[c] CCR abbreviates curved crown rump.
[1] d abbreviates day.

Fig. 1. Influence of nutrient restriction and arginine supplementation of ewes on offspring body weights over time.



CON abbreviates control diet, RES abbreviates restricted diet, RES-ARG abbreviates restricted supplemented with arginine diet

[+, ++, +++] Means within a day with different symbols differ

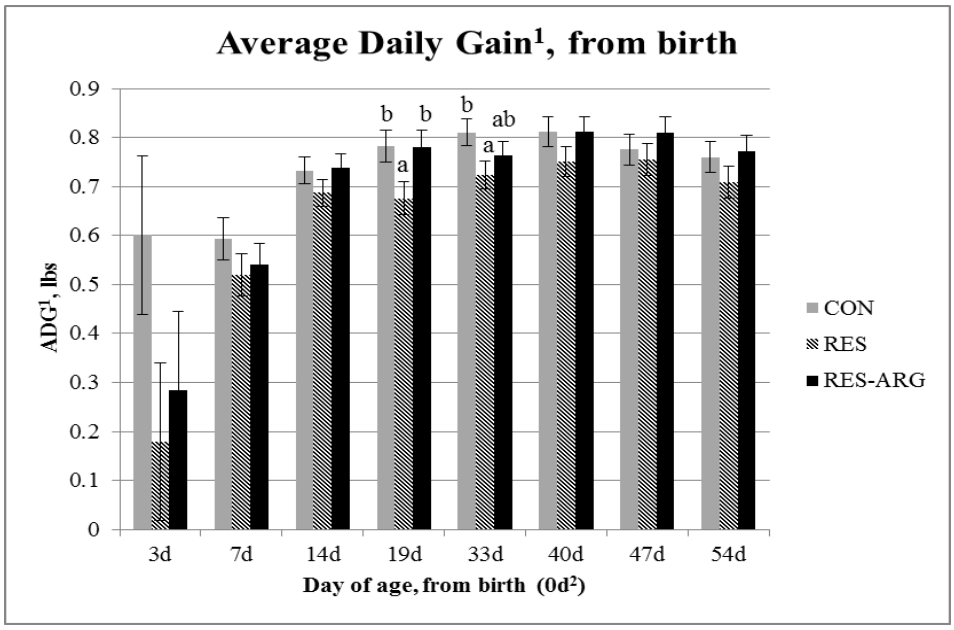
[+] = CON is significantly different from RES, RES-ARG is similar to both ($P < 0.05$)

[++] = RES is similar to RES-ARG, and significantly different from CON ($P < 0.05$)

[+++] = CON and RES-ARG are similar, and significantly different from RES ($P < 0.05$)

[1] d abbreviates day

Fig. 2. Influence of nutrient restriction and arginine supplementation to ewes on offspring average daily gains over time.



CON abbreviates control diet, RES abbreviates restricted diet, RES-ARG abbreviates restricted supplemented with arginine diet

[a,b] Means within a day with different superscripts differ ($P < 0.05$)

[1] ADG abbreviates Average Daily Gain

[2] d abbreviates day

FLOCK CALENDAR OUTLINE

The following guidelines are neither inclusive nor intended to fit every sheep operation. Each operation is different, therefore, each "calendar event" should be tailored to each flock's needs.

PRIOR TO BREEDING

1. Bag and mouth ewes and cull those that are not sound.
2. Replace culled ewes with top-end yearlings or ewe lambs.
3. Keep replacement ewes lambs on growing rations.
4. Evaluate sires:
    A. Be sure they are vigorous, healthy and in good breeding condition.
    B. Rams should be conditioned at least a month before breeding season. Flush rams in poor condition.
    C. Allow at least two mature rams (preferably three) or four buck lambs per 100 ewes.
5. Flush ewes:
    A. One pound grain/day two to five weeks before breeding (usually 17 days).
    B. If ewes are over-conditioned, the effect of flushing will be lessened.
6. Vaccinate ewes for vibriosis and enzootic abortion (EAE).
7. Identify all ewes and rams with ear tags, paint brands or tattoos.

BREEDING

1. The ovulation rate of a ewe tends to be lower at the first part of the breeding season. Vasectomized or teaser rams run with ewes through the first heat period tend to stimulate then and increase the ovulation rate at the second heat period.
2. Use a ram marking harness or painted brisket to monitor breeding. Soft gun grease with a paint pigment mixed in works well for painting the brisket. A color sequence of orange, red and black is recommended with colors being changed every 17 days.
3. Leave rams in NO LONGER than 51 days (35 days is more desirable).
    A. An exception may be with ewe lambs. Allowing them four cycles or 68 days may be beneficial.
4. Remove rams from ewes after the season (don't winter rams with ewes).

PRIOR TO LAMBING (First 15 weeks)

1. Watch general heath of ewes. If possible sort off thin ewes and give extra feed so they can catch up.
2. Feed the poor quality roughage you have on hand during this period, saving better for lambing.
3. An exception to the above is feeding pregnant ewe lambs. They should receive good quality roughage and grain (about 20 percent of the ration) during this period.

LAST SIX WEEKS BEFORE LAMBING

1. Trim hooves and treat for internal parasites.
2. Six to four weeks before lambing feed 1/4 to 1/3 pound grain/ewe/day.
3. Shear ewe before lambing (with highly prolific ewes at least a month before is preferred). Keep feeding schedule regular and watch weather conditions immediately after shearing (cold).
4. Vaccinate ewe for enterotoxaemia.
5. Control lice and ticks immediately after shearing.
6. Four weeks before lambing increase grain to 1/2 to 3/4 pound/ewe/day (usually done immediately after shearing.
7. Give A-D-E preparations to ewes if pastures and/or roughage are or have been poor quality.
8. Feed selenium-vitamin E or use an injectable product if white muscle is a problem. Caution DO NOT use both.
9. Check facilities and equipment to be sure everything is ready for lambing.
10. Two weeks before lambing increase grain to 1 pound/ewe/day.

LAMBING

1. Be prepared for the first lambs 142 days after turning the rams in with the ewe, even though the average pregnancy period is 148 days.
2. Watch ewes closely. Extra effort will be repaid with more lambs at weaning time. Saving lambs involves a 24-hour surveillance. Additional help at this time is money well spent.
3. Pen a ewe and lambs in lambing pen (jug) after lambing, not before.
4. Grain feeding the ewe during the first three days after lambing is not necessary.
5. Be available to provide assistance if ewes have trouble lambing.
6. Disinfect lamb's naval with iodine as soon after birth as possible.
7. Be sure both teats are functional and lambs nurse as soon as possible.
8. Use additional heat sources (heat lamps, ect) in cold weather.
9. Brand ewes and lambs with identical numbers on same side. Identify lambs with ear tags, tattoos or both.
10. Turn ewes and lambs out of jug as soon as all are doing well (one to three days).
11. Bunch up ewes and lambs in small groups of four to eight ewes and then combine groups until they are a workable size unit.
12. Castrate and dock lambs as soon as they are strong and have a good start (two days to two weeks of age). Use a tetanus toxoid if tetanus has been a problem on the farm (toxoids are not immediate protection, it takes at least ten days for immunity to build).
13. Vaccinate lambs for soremouth at one to two weeks of age if it has been a problem in the flock.
14. Provide a place for orphaned lambs. Make decision on what lambs to orphan as soon after birth as possible for best success. Few ewes can successfully nurse more than two lambs.

END OF LAMBING TO WEANING

1. Feed ewes according to the number of lambs sucking. Ewes with twins and triplets should receive a higher plane of nutrition.
2. Provide creep feed for lambs (especially those born during the winter and early spring).
3. Vaccinate lambs for overeating at five weeks and seven weeks of age.

WEANING

1. Wean ewes from lambs, not lambs from ewes. If possible, remove ewes from pen out of sight and sound of lambs. If lambs have to be moved to new quarters, leave a couple of ewes with them for a few days to lead the lambs to feed and water locations.
2. Lambs should be weaned between 50 and 60 days of age when they weigh at least 40 pounds and are eating creep and drinking water. The advantage of early weaning is that the ewe's milk production drops off to almost nothing after eight weeks of lactation.
2. Grains should be removed from the ewe's diet at least one week prior to weaning and low quality roughage should be fed. Restriction of hay and water to ewes following weaning lessens the chance of mastitis to occur. Poorer quality roughage should be fed to the ewes for at least 10-14 days following weaning.
3. Handle the ewes as little as possible for about 10 days following weaning. Tight udders bruise easily. If possible, bed the area where the ewes will rest heavily with straw to form a soft bed for the ewes to lay on.

WEANING TO PRE-BREEDING

1. If ewes go to pasture, treat for internal parasites.
2. Feed a maintenance ration to the ewes. Put ewe lambs that lambed back on a growing ration once they have quit milking.
3. Adjust ewes condition so they can be effectively flushed for next breeding season. Don't get ewes too fat prior to breeding.

REARING LAMBS ARTIFICIALLY (ORPHANS)- MANAGEMENT TIPS

Within 2 to 4 hours after birth, decide which lambs among those from multiple births you should remove. Look for the weaker, or smaller ones to choose for artificial rearing. It is important to make the decision early. Relatively weak lambs remaining with ewes can experience more stress than those reared artificially. Consider the following tips:

♦ It is essential that newborn lambs receive colostrums milk. Cow's colostrums will work if ewe's milk is not available. Do not dilute with water or warm too quickly if colostrums is frozen.

♦ Lambs should be removed from sight and hearing distance of ewes.

♦ Provide a warm, dry , draft-free area to start lambs.

♦ Use a good milk replacer that is 30% fat and at least 24% protein. Each lamb will require from 15 to 20 pounds of replacer to weaning.

♦ Lambs may require some assistance the first day or two to teach them to nurse on whatever feeding device is used.

♦ Start on nurser quickly, young lambs start easier.

♦ Self feed cold milk replacer after lambs are started. Milk replacers should be mixed with warm water for best results and then cooled down. Lambs feed cold milk well with less problems from scours and other digestive distrubances. Cold milk keeps better too.

♦ There is a Formaldehyde solution commercially avaliable that retards bacterial growth in milk (1cc/ gallon milk).

♦ Hang a light over the milk replacer feeding device and dry ration feeder.

♦ Avoid placing young lambs with older lambs, as they may be pushed aside and may not be able to obtain the milk replacer. Remember that lambs nursing ewes drink 25 to 40 times per 24 hours. Best results have been obtained when lambs are fed in groups of 3 to 4 initially. After lambs are successfully trained, they can be handled in groups of 25.

♦ Inject lambs in the first few days with Iron Dextran, Vitamin A-D-E, and Selenium-Vitamin E. At 15 days of age, vaccinate for overeating (Colostridum perfringen type C & D).

♦ Provide lambs with a high-quality creep feed as soon as possible. Provide ample fresh water in front of lambs at all times. Do not feed hay or oats the first three week after weaning, as it encourages bloat. Caution! Do not feed leafy alfalfa until two weeks after weaning, as it encourages bloat.

♦ Wean lambs abruptly at 21-30 days of age. When to wean depends upon whether lambs are eating creep feed and drinking water. Newly weaned lambs will go backwards for several days. Don't be alarmed, they will make compensating gains later on.

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