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Residual feed intake does not predict efficiency of limit-fed ewe lambs

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The objective of this research was to evaluate the performance of ewe lambs on a limit-fed ration that were divergently selected from high, medium, and low residual feed intake (RFI) groups. We did not find any difference in growth, feed efficiency, or tissue deposition among RFI grouped ewe lambs. Therefore, we caution the use of RFI values as indicators of efficiency in any setting other than the environment by which animals were tested.

INTRODUCTION

A reduction in feed intake without compromising biological or economic efficiency could have a significant positive impact on the sheep industry. The concept of developing an alternative feed efficiency measurement that is independent of growth traits was first proposed by Koch et al. (1963). Residual feed intake is the difference between actual feed intake and predicted feed intake based upon maintenance of BW and production by linear regression. Numerous research efforts have shown that there is considerable individual animal variation in RFI in cattle (reviewed by Archer et al., 1999 and Herd et al., 2003) and sheep (Snowder and Van Vleck, 2003 and Cammack et al., 2005). However, most RFI testing has been conducted post-weaning on medium-to-high energy diets and has been related to potential feed savings in the feedlot (Snowder and Van Vleck, 2003). Research from our laboratory found no phenotypic correlation between RFI that was determined on a pelleted grower ration and RFI from lambs fed a chopped hay diet at maintenance (Redden, unpublished); however, we could not attribute the lack of RFI relationship to diet or rate of growth. Therefore, our objective was to measure the production differences of ewes previously been determined to be highly efficient (low RFI) or highly inefficient (high RFI) when limit-fed the same diet.

PROCEDURES

Targhee ewe lambs (n = 49) were selected randomly from the Red Bluff Research Ranch 2009 spring-born lamb crop. Use of animals was approved by Montana State University Animal Care and Use Committee.

Determination of RFI. On January 28, 2010, a 49-d experiment was conducted to determine RFI during active growth using the GrowSafe feed intake system (GrowSafe Systems Ltd., Airdrie, AB, Canada). Ewes were housed together in one pen (30 X 30 ft) with 4 GrowSafe pods at the Montana State University Nutrition Center. Elevated platforms and false bottoms were constructed to modify GrowSafe beef cattle stanchions and feed bunks, respectively, for sheep. Ewes

were given ad libitum access to a pelleted grower diet (75% TDN, 16% CP) and water. After a 2 week acclimation period, individual feeding events and feed disappearance recordings were initiated. Feed samples were collected weekly and stored for later analysis. Ewe BWs were measured weekly with two consecutive day weights recorded at the start and end of the experimental period. Growth rates of individual ewes were modeled by linear regression of 7-d BW by using a PROC GLM procedures of SAS (SAS Inst. Inc.), and regression coefficients were used to compute ADG, initial and final BW, and metabolic BW (**MBW**; midtest $BW^{0.75}$) as described by Lancaster et al. (2009). Ewe RFI was calculated for each individual as the difference between actual feed intake and expected feed intake. Expected feed intake was calculated by regressing the actual feed intake against MBW and ADG during the trial (Koch et al., 1963). To further characterize RFI, ewes were classified into low, medium, and high RFI groups that were <0.5, ±0.5, and >0.5 SD, respectively, from the mean RFI.

Limit-Fed Experiment. After the conclusion of the RFI determination experiment, ewes were removed from the GrowSafe testing facility and limit fed for 35 d. Twelve ewes per RFI grouping were selected for the limit-fed experiment. Ewe lambs that had the largest negative, largest positive and closest to zero RFI were assigned to low, high, and medium RFI groups, respectively. Three ewes from a RFI group were placed in each pen (12 x 48 ft). Twelve feedlot pens were used in this experiment and pen was the experimental unit. Ewes were fed twice daily at a rate that NRC (2008) predicted gain of 0.10 lb/d. Ewe BW were measured weekly with two consecutive day weights recorded at the start and end of the experimental period. Growth rates of individual ewes were modeled by linear regression of 7-d BW as described previously. Rib-eye area and backfat depth were determined at the beginning and end of the limit-fed experiment to estimate lean and fat deposition.

RESULTS AND DISCUSSION

Figure 1 illustrates the diversity in residual feed intake of ewes tested in this experiment. Black, blue, and red bars are the high, medium, and low efficiency groups, respectively. During the RFI determination experiment, ewes in the low-RFI group consumed 16% less feed ($P < 0.01$) than ewes in the high -RFI group, while ewe MBW and ADG were similar among RFI groups (Table 1). A similar percent in feed reduction between high- and low-RFI groups in beef cattle was reported by Lancaster et al. (2009).

Limit-fed Experiment. There were no detectable differences ($P > 0.19$) among RFI groups for average daily gain, rib-eye area growth, or back-fat deposition (Table 1). Similarly, Redden et al. (unpublished) found that RFI determined on a grower pelleted diet did not predict RFI determined on a chopped hay roughage diet. In general, an RFI difference among animals has been theorized to be a reduction in maintenance, growth requirements, or both. However, it appears that RFI is a measure of intake differences rather than physiological status of an animal. Therefore, we conclude that once ewe lambs are removed from an ad libitum diet RFI no longer predicts ewe lamb efficiency.

IMPLICATIONS

Measuring residual feed intake has the potential to significantly reduce production costs of livestock operations. However, producers must be aware that RFI should not be used to estimate efficiency savings in production settings other than those similar to the test environment. This research warrants further investigation of the relationship between RFI and efficiency savings in settings other than the RFI test.

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Table 1. Relationship between RFI determined efficiency groups on limit-fed ewe lamb performance

Item[1]	RFI Groups – Hay Diet (Exp. 2)			SE	*P*-value
	High Efficiency	Medium Efficiency	Low Efficiency		
No. of ewes	12	12	12		
RFI Traits[2]					
RFI, lb/d	0.44	0.00	-0.44	0.04	<0.01
ADG, lb/d	0.65	0.66	0.63	0.04	0.78
BW, lb	113	113	111	0.62	0.73
DMI, lb/d	4.72	5.07	5.55	0.15	<0.01
Limit-Fed Performance[3]					
No. of pens	4	4	4		
DMI, lb/d	2.9	3.0	2.9		
ADG, lb/d	0.08	0.07	0.07	0.02	0.86
REA, in^2					
Initial	2.12	2.05	2.16		
Final	2.71	2.74	2.73		
Change	0.59	0.70	0.57	0.31	0.19
BF, in					
Initial	0.18	0.18	0.19		
Final	0.22	0.23	0.25		
Change	0.04	0.06	0.07	0.01	0.39

[1]RFI = residual feed intake; ADG = average daily gain; BW = body weight; DMI = dry matter intake; REA = rib-eye area; BF = back fate depth.
[2]RFI traits were determined during a 49 d ad libitum feeding trial and feed intake data was collected via GrowSafe Technologies.
[3]Limit-fed performance traits were determined during a 35 d feeding trial. Ewes were fed the same diet at was fed during the RFI trial; however, they were limit-fed to gain BW at 0.1 lb per day.

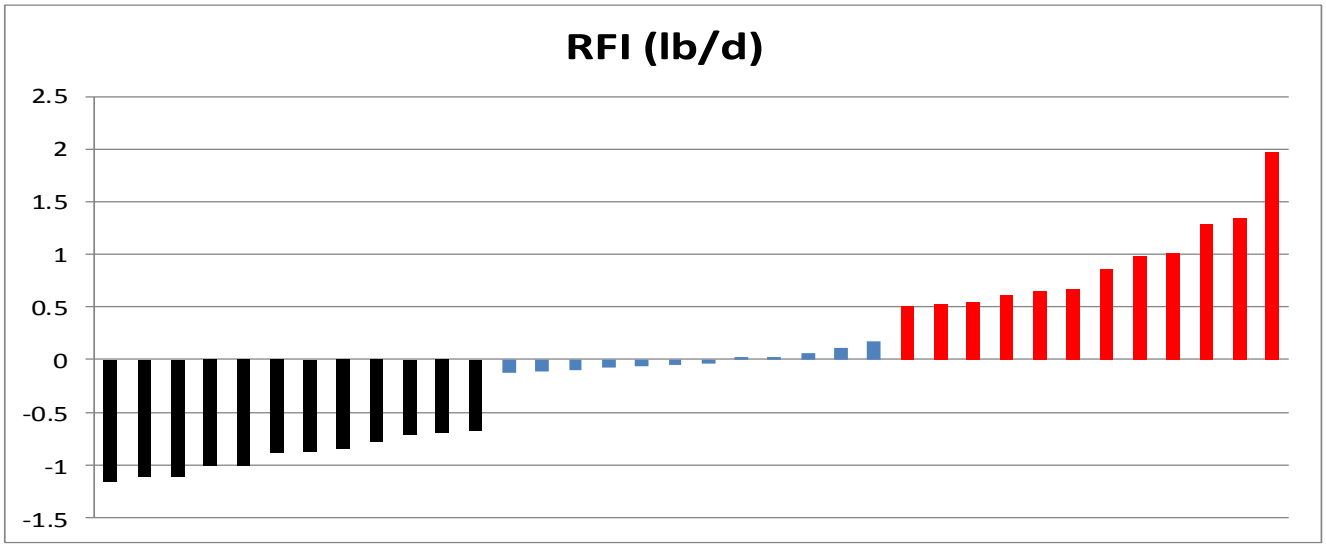

Figure 1. Distribution of residual feed intake (RFI) of ewes ranked from lowest to highest. Each ewe's RFI (kg) value is represented by a bar. Black, blue, and red bars are the high, medium, and low efficiency groups, respectively.

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Effects of graded levels of zeranol implants on feedlot performance, carcass characteristics, and incidence of prolapse and mortality in lambs[1]

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The objective of this research was to determine the feasibility of implanting feedlot lambs with increasing amounts of zeranol. Previous research demonstrated increased growth performance in lambs implanted with zeranol, but also indicated a risk for increased incidence of prolapse resulting in increased mortality. If lamb feedlot operations could increase average daily gain and decrease days on feed, considerable increases in profitability could be obtained.

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SUMMARY

The objective of this research was to compare the growth performance and carcass characteristics of feedlot lambs implanted with four levels of zeranol. One hundred forty four crossbred lambs (65 ± 10 lbs) were placed into sixteen feedlot pens and finished according to treatment in a 116 day finishing study. Treatments included: 1) **0** (no implant), 2) **12** (12 mg zeranol implant), 3) **24** (24 mg zeranol implant), and 4) **36** (36 mg zeranol implant). Lambs were implanted with zeranol (Ralgro®, Schering-Plough) according to treatment on day 0. All treatments received the same 84.7% corn and 15.3% market lamb pellet (DM basis) ration ad libitum. The feedlot study ended on d 116, and lambs were harvested day 118. Carcass data was collected 24 hr post-chill. There were no differences between treatments for body weight, ADG, DMI, and G:F ($P \geq 0.33$). Carcass characteristics also were not affected by treatment ($P \geq 0.07$). However, 24 and 36 treatment groups had increased incidence of prolapse ($P = 0.03$) compared to lambs implanted with 0 and 12 mg. Lambs from treatment groups 24 and 36 also had increased percent mortality ($P = 0.04$) compared to 0 lambs, with 12 being intermediate. No differences ($P \geq 0.07$) between treatments for growth and carcass characteristics were observed. The increased cost and labor associated with implanting lambs and treating prolapses, as well as the monetary loss from lamb death, indicate implanting lambs with zeranol is economically impractical.

INTRODUCTION

Zeranol has been shown to improve growth performance in lambs when implanted with 12 mg once (Field et al., 1993; Salisbury et al., 2007; and Stultz et al., 2001) or more than once (Hufstedler et al., 1996 and Nold et al., 1992). Most research indicates zeranol does not alter carcass characteristics (Arnsperger et al., 1976; Hutcheson et al., 1992; Olivares and Hallford, 1990; and Salisbury et al., 2007), although some studies report conflicting results (Field et al., 1993; Stultz et al., 2001; and Wiggins et al., 1979). Zeranol has also been

implicated in increased incidence of prolapse (Arnsperger et al., 1976 and Salisbury et al., 2007), resulting in decreased use of zeranol in the United States. However, it has been estimated that as many as half of market lambs fed in Mexico are implanted with zeranol (G. Amaya, 2010). Research by Eckerman et al. (2010) compared lambs raised conventionally and implanted with 36 mg zeranol to lambs managed using naturally raised guidelines. Results showed conventional lambs had increased growth performance, but also had increased incidence of prolapse and mortality.

Our objective for this study was to determine the effects of graded amounts of zeranol on lamb feedlot performance and carcass characteristics, as well as incidence of prolapse and mortality. The hypothesis tested was lambs implanted with greater amounts of zeranol would have improved growth performance, without altering carcass quality or increasing incidence of prolapse or mortality.

PROCEDURES

Animal Management and Treatments. All experimental protocols were approved by the North Dakota State University Animal Care and Use Committee. At two weeks of age, tails were docked, males castrated, and all lambs were vaccinated for clostridium perfringens types C and D, as well as for tetanus (Bar Vac CD-T, Boehringer Ingelhein, Ridgefield, CT). Lambs were vaccinated with CD-T again at 60 d of age and d -1 of the study. One hundred forty four spring-born crossbred lambs (wethers and ewes, 65 ± 10 lbs) were stratified by weight and sex. Within stratification, lambs were assigned randomly to treatment: 0, 12, 24, or 36 mg zeranol implant. Treatments were applied in a completely randomized design to evaluate the outlined objectives.

Lambs were implanted according to treatment on d 0 with Ralgro® (Schering-Plough Animal Health Corp., Union, NJ). Lambs were then moved to 16 feedlot pens (n = 4). Each pen represented one experimental unit and contained 9 lambs. Lambs were offered feed ad-libitum via bulk feeders and had continuous access to clean, fresh water. Lambs had access to shade and were observed daily to monitor health. All lambs received the same ration: 84.7% corn and 15.3% market lamb pellet (DM basis, Table 1). Lambs were treated with antibiotics as necessary. Lambs which rectally or vaginally prolapsed were treated using techniques best-suited for each situation, including the use of sutures, oxytetracycline, and general antibiotics.

Experimental Periods and Sampling Procedures. The study was divided into four periods, consisting of 28, 28, 26, and 34 d, respectively. Lambs were weighed two consecutive days at initiation (d -1 and 0) of the trial and after the third and fourth period. Single day weights were taken on d 28 and 56, with two day weights taken d 81 and 82 as well as d 115 and 116. Thirty lambs minimum 140 lbs) were harvested on d 84 at Iowa Lamb Corporation in Hawarden, IA. Ninety six lambs (minimum 125 lbs) were harvested on d 118 at Iowa Lamb Corporation. Carcass data were collected 24 h post chill by trained university personnel.

Bulk feeders were emptied at the end of each period, with weight and samples of refusals collected to determine period DMI. Ration and feed ingredient samples approximately 0.44 lb) were collected every 28 d, dried at 55°C for 48 h to determine DM, and analyzed for ADF, NDF, N, and OM at the North Dakota State University Animal Science Nutrition Laboratory.

Statistics. Lamb performance data were analyzed as a completely randomized design using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) with pen serving as experimental unit. Carcass data were analyzed with missing data points from underweight lambs not included in the data set, with pen serving as experimental unit. Repeated measures was used to analyze period effects for BW, ADG, G:F, and DMI. The model included treatment, day, and day x treatment interaction. The covariance structure used was First Order Antedependence. Other structures were tested but First Order Ante-dependence was the best fit. Data are presented as least squares means with differences

considered significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

No differences were observed between treatments for body weight gain or ADG (Table 2, $P \geq 0.64$). Feed intake and feed efficiency were not different between treatments ($P \geq 0.33$). This is similar to some of the previous research that found no effect of zeranol implant on ADG (Field et al., 1993; Nold et al., 1992; and Wiggins et al., 1976) or feed efficiency (Wiggins et al., 1976). However, the majority of the research indicates implanting feedlot lambs with 12 mg zeranol increases ADG (Arnsperger et al., 1976; Hutcheson et al., 1992; Salisbury et al., 2007; Stultz et al., 2001; Wiggins et al., 1979; and Wilson et al., 1972) and feed efficiency (Field et al., 1993; Olivares and Hallford, 1992; and Stultz et al., 2001). Previous research also indicates feed efficiency can be improved by implanting lambs twice with 12 mg zeranol (Nold et al., 1992) and both feed efficiency and ADG can be improved by implanting lambs with 12 mg of zeranol three or more times (Hufstedler et al., 1996). However, Hufstedler et al. (1996) also observed decreased dressing percentage and increased carcass maturity in lambs repeatedly implanted.

There were no differences ($P \geq 0.07$) in carcass characteristics in lambs implanted with graded levels of zeranol. This is in accordance with most research, although some studies have indicated zeranol can alter carcass characteristics. Stultz et al. (2001) observed increased carcass weights in lambs implanted with 12 mg of zeranol compared to control lambs, most likely resulting from increased live weights at the termination of the study. It was also observed that implanted lambs had increased ribeye area compared to nonimplanted lambs. Field et al. (1993) examined the effects of 12 mg zeranol implants in rams and wethers, and found zeranol caused increased fat depth in implanted wethers. However, no other differences in carcass characteristics were observed. Another study analyzing the effects of re-implanting lambs found lambs implanted at birth and weaning, and lambs implanted at 55 and 98 d of age, had increased leg score compared to lambs that received no implants (Nold et al., 1992). However, Wiggins et al. (1979) observed decreased dressing percentage in implanted lambs, which was a factor of increased gastrointestinal tract weight. Again, no other carcass characteristics were significantly altered by the use of 12 mg zeranol implants. The aforementioned research would suggest that while zeranol could potentially alter carcass characteristics, the effects are minimal.

The major effects observed in the present study were the increased percent prolapse ($P = 0.03$) in the 24 and 36 mg implant groups compared to the 12 mg and control group. The increased percent prolapse subsequently resulted in increased percent mortality ($P = 0.04$) in 24 and 36 treatments compared to 0 treatment lambs. This high incidence of morbidity likely caused lambs implanted with 24 and 36 mg zeranol to have decreased growth performance compared to what was expected. Lambs that were treated for prolapse often went off feed and gained little or no weight. Only two of the previously mentioned studies (Salisbury et al., 2007 and Arnsperger et al., 1976) reported complications of

Table 1. Ingredient and nutritional composition of diet fed to feedlot lambs

Item	Diets
Ingredient	DM basis
Whole Corn, %	84.7
Market Lamb Pellet[1], %	15.3
Nutrient composition	
CP, %	13.12
Ash, %	4.59
NDF, %	13.47
ADF, %	3.41

[1]Market Lamb Pellet contained: 0.22 g/kg chlortetracycline, 38% CP, 4.25% Ca, 0.6% P, 3.5% salt, 1.2 mg/kg Se, 52,920 IU/kg Vitamin A, 5,292 IU/kg Vitamin D, and 209 IU/kg Vitamin E.

Table 2. Effects of graded levels of zeranol on lamb growth performance, carcass characteristics, and health

Item	Treatment[1]				SEM[2]	*P* – value[3]
	0	12	24	36		
Body Weight[4], lb						
d 0	65	65	66	66	2.2	0.81
d 28	88	89	89	91	2.2	0.79
d 56	109	109	108	111	2.2	0.83
d 84	128	126	124	125	2.2	0.57
d 112	142	143	143	145.5	2.2	0.98
ADG[5], lb·d^{-1}	0.74	0.73	0.71	0.67	0.04	0.52
Intake[6], lb DM·hd^{-1}·d^{-1}	3.75	3.70	3.84	3.84	0.11	0.94
G:F[7]	0.22	0.22	0.21	0.20	0.01	0.33
HCW, lb	74.9	74.1	73.6	73.2	1.28	0.81
Leg Score[8]	11.8	12.0	12.0	12.3	0.18	0.31
Conformation Score[8]	11.5	12.0	12.0	12.0	0.14	0.07
Fat Depth, in[9]	0.98	0.98	1.08	1.03	0.03	0.14
Body Wall Thick, in	0.29	0.30	0.33	0.30	0.02	0.62
Ribeye Area, in^2	2.75	2.68	2.53	2.55	0.08	0.17
Flank Streaking[10]	378.75	355.75	376.25	352.00	10.94	0.25
Quality Grade[8]	12.3	12.0	12.0	12.0	0.1	0.43
Yield Grade[11]	3.25	3.38	3.70	3.40	0.24	0.62
BCTRC, %[12]	45.58	45.43	44.70	45.05	0.36	0.35
Dress, %	50.73	50.73	50.83	50.25	0.40	0.74
Prolapse, %	2.78^a	5.55^a	24.98^b	27.75^b	6.31	0.03
Mortality, %	0.00^a	5.55^{ab}	11.10^b	13.88^b	3.10	0.04

[1]Treatments: 0 (0 mg zeranol implant), 12 (12 mg zeranol implant), 24(24 mg zeranol implant), 36 (36 mg zeranol implant).

[2]Standard Error of Mean; n = 4.

[3]*P* – value for F-tests of mean.

[4]*P*-values for body weight TRT (*P* = 0.90), Pd (*P* < 0.001) TRT x Pd (*P* = 0.49).

[5]*P*-values for ADG TRT (*P* = 0.52), Pd (*P* < 0.001) TRT x Pd (*P* = 0.61).

[6]*P*-values for Intake TRT (*P* = 0.94), Pd (*P* < 0.001) TRT x Pd (*P* = 0.71).

[7]*P*-values for G:F TRT (*P* = 0.33), Pd (*P* < 0.001) TRT x Pd (*P* = 0.24).

[8]Leg score, conformation score, and quality grade: 1 = cull to 15 = high prime.

[9]Adjusted fat depth and yield grades.

[10]Flank streaking: 100-199 = practically devoid; 200-299 = traces; 300-399 = slight; 400-499 = small; 500-599 = modest.

[11]Yield Grade = 0.4 + (10 x adjusted fat depth, in).

[12]Boneless closely trimmed retail cuts, % = (49.936 – (0.0848 x Hot Carcass Weight, lb.) - (4.376 x Fat Depth, in.) – (3.53 x BW, in.) + (2.456 x Ribeye Area, in^2)).

prolapse resulting from zeranol implants. Even the studies examining the use of 2, 3, or more implants did not report any incidences. Annotative information suggests as much as 50% of Mexican feeder lambs are implanted with zeranol (G. Amaya, 2010), but feedlot operations do not experience the rate of prolapse that was observed in the present study. However, research by Arnsperger et al. (1976) resulted in increased incidence of rectal and vaginal prolapses in lambs raised on feedlot rations. The high incidence of prolapse in the study by Arnsperger et al. (1976) did not appear to hinder the growth performance of the lambs, as we believe the case to be in the present study. Despite the prolapses, implanted lambs in studies by Arnsperger et al. (1976) still had increased ADG compared to nonimplanted lambs. Another study observed 5 and 20% percent vaginal prolapse in lambs implanted once and twice, respectively, with control lambs having no prolapses (non-significant, Salisbury et al., 2007). However, in that study implanted lambs still had increased ADG and feed efficiency compared to control lambs.

The rations of the studies by Arnsperger et al., (1976) and Salisbury et al. (2007), as well as the present study, utilized high concentrate feedlot rations. In contrast, many Mexican feedlots use lower concentrate rations (G. Amaya, 2010), which could be the cause of the decreased incidence of prolapse. Arnsperger et al. (1976) noted that lambs implanted with zeranol and raised on pasture did not experience the high rate of prolapse observed in the feedlot lambs. Salisbury et al. (2007) hypothesized that the combination of zeranol causing uterine muscle contraction and grain finishing causing increased fat deposition around the tail led to the increased incidence of vaginal prolapse. As such, high concentrate diets could be implicated in contributing to increased incidence of prolapse.

IMPLICATIONS

The increased incidence of prolapse and mortality observed in lambs implanted with 24 or 36 mg of zeranol, without increased growth performance or carcass characteristics, indicate the use of high doses of zeranol implants are not practical on conventional lamb feedlot settings that utilize high concentrate rations. The increased labor associated with implanting lambs and treating prolapses, in addition to the cost of implants and death loss, prevent the use of zeranol implants from being economically feasible in lamb feedlot operations. However, there is the possibility that zeranol could be beneficial to producers raising lambs on pasture or range. Zeranol could provide improved growth performance to lambs that would otherwise underperform on lower quality forage. This aspect of zeranol use should be further examined, but the use of zeranol implants in feedlot lambs is not recommended.

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Impacts of arginine on ovarian function and reproductive performance at the time of maternal recognition of pregnancy in ewes

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Reproductive performance is the largest determinant of income in the sheep flock. The objective of the current study was to determine if supplementation with the amino acid arginine surrounding the time of maternal recognition of pregnancy enhances ovarian function and reproductive performance in ewes. Recently arginine supplementation strategies have proven to be a suitable method to enhance reproductive loss in livestock creating a more profitable enterprise for the producer.

INTRODUCTION

As a precursor for nitric oxide, polyamines, and proteins, the amino acid arginine plays a vital role in metabolism and reproduction (Wu and Morris, 1998). Supplemental arginine has been reported to increase the number of live piglets born per sow (Mateo et al., 2007). Furthermore, pregnant rats supplemented with arginine throughout gestation exhibited an increase in embryonic survival and litter size (Zeng et al., 2008). Recently at NDSU we observed increased ovarian blood flow, serum progesteron and fetal number, despite similarities in ovulation rate, in ewes injected with L-arginine during the first 15 d post-breeding. Collectively, these studies suggest that reproductive efficiency can be enhanced via supplementation with arginine.

In sheep, embryonic and fetal deaths during pregnancy account for 25 to 50% of the total number of fertilized ova (Dixon et al., 2007). Most embryonic loss has been reported to occur before d 18 (Quinlivan, 1966). Only a small percentage of embryos are inherently non-viable in the ewe (Wilmut et al., 1986), which would suggest that the majority of early embryonic losses can be prevented.

Communication between the embryo and the maternal system must be established following conception to ensure normal development of the embryo. Maternal recognition of pregnancy in sheep occurs around d 13 following ovulation. During this critical period, the conceptus elongates from a blastocyst to a filamentous form, which produces interferon tau that is responsible for preventing the development of the endometrial luteolytic mechanism (Spencer and Bazer, 2002). The presence of interferon tau allows for maintenance of the CL, which is the primary structure responsible for progesterone production during early pregnancy in sheep.

The objective of this study was to determine the effects of arginine supplementation surrounding the time of maternal recognition of pregnancy on ovarian

hemodynamics, early reproductive loss and lamb birth weight in Rambouillet ewes.

PROCEDURES

Rambouillet ewes of a similar body weight and age were randomly assigned to one of two groups: control (**CON**; n = 47) and L-arginine (**ARG**; n = 47). All ewes received a CIDR device for 12 d. Following CIDR removal a single injection of PG -600 was given to help initiate follicular development and ensure ovulation. Thereafter, ewes were exposed to fertile rams. From d 9 to d 14 post-estrus ewes received L-arginine HCl (equivalent to 27 mg of L-arginine/kg of BW) or saline (CON) intravenously once daily. Daily blood samples were obtained (n = 10 ewes/ subgroup) immediately after treatment (0 h) to assess progesterone (P4) concentrations and at -0.5, 0, 0.5, 1, 2, 4, 6 and 8 h on d 10 to determine circulating concentration of arginine in response to treatment. Ovarian hemodynamics (d 12) was determined with color Doppler ultrasonography (n = 10 ewes/ subgroup) and reproductive losses (d 25, 45 and 65; n = 94) were determined using B-mode ultrasonography techniques.

RESULTS

On d 10 of pregnancy, serum concentrations of arginine (nmol/mL) were elevated in a subset of ewes (n=10 ewes/ treatment group) ewes injected with arginine vs. CON ewes at 0 ($P < 0.001$), 0.5 ($P < 0.001$), 1 ($P < 0.001$), 2 ($P < 0.001$) and 4 h ($P < 0.001$), but were similar ($P \geq 0.70$) at -0.5, 6, and 8 h (Figure 1). Metabolites of arginine, ornithine and citrulline were measured. On d 10, ornithine levels were elevated in ARG vs. CON ewes at 0.5 ($P < 0.03$), 1 ($P < 0.001$), 2 ($P < 0.001$), 4 ($P < 0.001$), 6 h ($P < 0.001$) and 8 h ($P < 0.02$). However there was no effect on circulating serum citrulline concentration ($P \geq 0.09$).

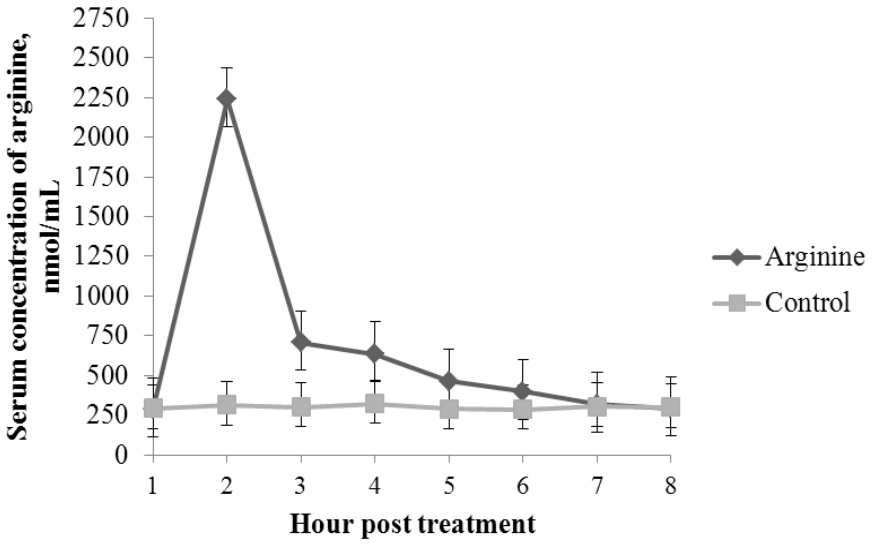

Figure 1. Effects of injectable L-arginine on serum arginine concentration (nmol/ mL) on Day 10 in Rambouillet ewes (**$P = 0.001$; *$P < 0.001$) from d 9 to 14 of the estrous cycle.

Carotid artery and ovarian hemodynamics were measured on d 12 with Doppler ultrasonography on a subset of ewes (n=10 ewes/treatment group). There were no differences in pulsatility index in those ewes treated with arginine vs. control in the ovarian hilus ($P \geq 0.49$). When measuring the vasculature surrounding the CL, there was no effect of arginine treatment compared with control ($P \geq 0.51$). Similar to the pulsatility index, resistance index was also not influenced with arginine treatment in the ovarian hilus or in the CL ($P \geq 0.49$ and $P \geq 0.51$; respectively). Despite similarities in CL in the subset of ewes blood sampled (ARG; 1.69 ± 0.12 and CON; 1.67 ± 0.16 CL/ewe; $P > 0.05$), CON ewes had greater serum progesterone concentration (ng/mL) compared with ARG on d 9 (6.11 ± 0.27 vs. 5.30 ± 0.15 respectively; $P < 0.02$) and 10 (6.50 ± 0.40 vs. 5.06 ± 0.21 respectively; $P < 0.005$) but similar for the remaining treatment period ($P \geq 0.06$) .

Treatment with arginine influenced pregnancy rate (ARG, 55%, n=47 and CON, 30%, n=47; $P \leq 0.02$), despite treatment similarities in pregnant ewes in CL number at d 25 in ARG ewes vs. CON (Table 1). As pregnancy progressed to d 45, a similar number of embryos were observed between ARG and CON ewes, with pregnancy rate remaining greater in ARG (ARG, 47% vs. CON, 26%; $P \leq 0.03$; Table 1). By d 65 of pregnancy, the ewes continued to maintain similar embryos in ARG ewes vs. CON (Table 1). Pregnancy rate was also greater

Table 1. Effects of L-arginine on number of corpora lutea and embryos

	Arginine[a]			Control[b]			
Item/ewe	Mean	SEM	n	Mean	SEM	n	*P*-value[c]
No. Corpora lutea	1.69	0.12	26	1.67	0.13	15	0.42
No. Embryos d 25	1.62	0.12	26	1.53	0.16	15	0.33
No. Embryos d 45	1.45	0.14	22	1.50	0.15	12	0.41
No. Embryos d 65	1.50	0.14	22	1.45	0.16	11	0.42

[a] Arginine, 27 mg/kg BW injectable arginine.
[b] Control, saline.
[c] *P*-value for F-test for treatment.

($P \leq 0.02$) at d 65 in ARG (47%) compared with CON (23%) ewes.

A total of 32 (n=47) and 16 (n=47) lambs were born from ewes exposed to rams on synchronized estrous in CON vs. ARG, respectively. Ewes treated with ARG gave birth to a similar number of lambs when compared with CON (1.78 ± 0.17 vs. 1.60 ± 0.27 respectively; $P < 0.58$). Average lamb birth weights (lb) did not differ between treatment groups (8.5 ± 0.44 vs. 9.0 ± 0.58 respectively; $P < 0.24$).

DISCUSSION

In the present study, pregnancy rate was greater in those ewes treated with injectable arginine when compared to control ewes. However, the overall pregnancy rates were lower than expected throughout the study. Ewes utilized were bred out of season and only those whom were mounted by a fertile ram following the synchronized estrous period were used for this study. This may be a justifiable reason for the overall low pregnancy rates across treatment groups.

In the current study, ewes treated with injectable L-arginine surrounding the time of maternal recognition of pregnancy (d 9 to 14) had enhanced pregnancy rates, increased circulating serum arginine and ornithine concentration, and elevated vascular resistance in peripheral blood flow. Arginine is important for many biological functions, including the synthesis of nitric oxide (Gouge et. al., 1998; Manser et. al., 2004). It may be reasonable to hypothesize 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. Nitric oxide and polyamines may have directly enhanced embryonic cellular proliferation and differentiation to ensure proper embryonic survival.

Progesterone is important for histotropic nutrition of the early embryo and suppression of the luteolytic mechanism (Lamming et al., 1989). In the present study, ewes treated with arginine had lower concentrations of progesterone relative to control ewes on d 9 and 10 of gestation, but were similar for the remaining treatment period. The lower levels of progesterone in arginine treated ewes may be due to an increase in metabolic clearance rate of steroids within the liver. Several studies have shown that low levels of progesterone can lead to a greater incidence of embryonic loss in sheep, and ultimately result in decreased ewe productivity (Dixon et. al., 2007). However, this finding was not observed in the current study. In fact, ARG treated ewes actually had greater pregnancy rates when compared to CON ewes.

IMPLICATIONS

In summary, treatment with arginine surrounding the time of maternal recognition of pregnancy may have prevented pregnancy loss in some ewes. Despite similarities in total CL and embryo numbers, overall pregnancy rate was increased in ewes treated with arginine. The enhanced pregnancy rate may have been due to arginine supplementation creating a more ideal uterine environment for the maintenance of embryos. During the early stages of embryogenesis, the supplemental arginine could have rescued weaker embryos entering the early stages of regression through its role in nitric oxide and polyamine synthesis. Nitric oxide and polyamines may have directly enhanced embryonic cellular proliferation and differentiation to ensure and promote proper embryonic survival.

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Effects of maternal metabolizable protein supplementation during the last 50 days of gestation on ewe and offspring performance and carcass characteristics[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 offspring performance and carcass characteristics.

SUMMARY

Supplementation of energy and/or protein to ruminants during gestation has become vital in programming the fetus for increased growth and performance throughout life. Therefore, we hypothesized that ewes on the restricted metabolizable protein (MP) diets would have reduced performance during the last 50 days of gestation, and therefore would have smaller lambs at birth. Secondly, we hypothesized that the lambs from ewes maintained on the restricted MP diets would have reduced feedlot performance and carcass characteristics. Two hundred ninety-five multiparous ewes were stratified by weight, body condition score, and expected lambing date into one of three dietary treatments: CON: 100% of the MP requirement, MED: 80% of CON, and LOW: 60% of CON (NRC, 2007) during the last 50 days of gestation. Ewe initial BW, final BW pre-partum, change in BCS during gestation, and BW change at lambing were not different ($P \geq 0.26$) between dietary treatments. Ewes maintained on the CON and MED diets had a greater ($P < 0.0001$) increase in BW change compared with the ewes maintained on the LOW diet. At lambing, the LOW and MED ewes had a greater ($P = 0.007$) reduction in change in BCS compared with the CON ewes. Three hour milk production and lamb weaning weight were not affected ($P \geq 0.65$) by ewe maternal MP supplementation. Maternal dietary treatment did not affect ($P \geq 0.13$) initial BW, final BW, ADG, feed efficiency, carcass weight, dressing percentage, LM area, back fat thickness, body wall thickness, leg score, conformation score, flank streaking, quality grade, and yield grade of wethers in the feedlot. Wethers from ewes on the LOW treatment had increased ($P = 0.01$) DMI compared with wethers from ewe on the MED treatment. The wethers from ewes supplemented with CON tended ($P = 0.10$) to have decreased days on feed in the feedlot compared with wethers from ewes supplemented with MED. Percent boneless, closely trimmed, retail cuts was increased ($P = 0.04$) in wethers from ewes maintained on the CON diet compared with wethers from ewes fed the LOW and MED diets. The tendency for wethers from the CON fed ewes to have reduced days on feed as

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well as the increased percentage of retail cuts suggests that those wethers were programmed in utero to have increased performance and carcass characteristics. These data suggest that ewes fed at 60% of MP requirements can still maintain pregnancy by becoming more efficient in partitioning nutrients to the fetus and mobilizing body reserves.

INTRODUCTION

Crude protein is supplemented during late gestation to cows and ewes to maintain body condition in dams as well as dam body weight (Martin et al., 2007; Swanson et al., 2008). By maintaining body weight and body condition the dam has more nutrient reserves to be utilized in maintaining pregnancy and growth of the fetus. However, very little research has evaluated supplementation of metabolizable protein (MP) during late gestation. Gestation length has also been shown to be reduced in cows fed severely restricted diets during early gestation compared with cows supplemented with adequate energy and CP (Long et al., 2010).

In many studies, birth weight was found not to be affected by dam supplementation or nutrient restriction (Anthony et al., 1986; Long et al., 2010; Martin et al., 2007; and Stalker et al., 2006). However, Swanson et al. (2008) observed that ewes fed diets meeting 60 and 140% of requirements of the early gestational ewe gave birth to heavier lambs than those ewes fed a diet meeting 100% of requirements of the early gestational ewe. Although birth and weaning BW was not significant due to maternal dietary restriction, those steers from cows fed a restricted diet had increased BW at the beginning and end of the feedlot phase (Long et al., 2010). However, these increased BW did not carry over into the carcass characteristics (Long et al., 2010). However, Ford et al. (2007) observed an increase in ultrasonography back fat in lambs from ewes fed a restricted diet during early to mid-gestation, once again yielding conflicting results on the effect of maternal supplementation during late gestation on progeny performance.

Therefore, we hypothesized that ewes on a restricted MP diets would have reduced performance during the last 50 days of gestation, and therefore would have smaller lambs at birth. We also hypothesized that the lambs from ewes maintained on the restricted MP diets would have reduced feedlot performance and carcass characteristics.

PROCEDURES

Ewes. Two hundred ninety-five multiparous ewes were stratified by weight, body condition score, and expected lambing date into one of three dietary treatments (Table 1): LOW: 60% of CON; MED: 80% of CON; and CON: 100% of the MP requirements of a ewe baring twins during the last 4 weeks gestation (NRC, 2007). Ewes were moved into a total confinement barn with a total of 21 pens (7 pens/dietary treatment) and acclimated to the CON diet for 7 d prior to starting dietary treatments. Ewes were supplemented with their respective treatment once daily at 0800 h. Supplementation was determined by the average body weight of the pen and fed according to NRC (2007) requirements. Ewes were given two hours to consume the supplement then low-quality forage (2.92% CP and 60.00% TDN; fescue hay) was offered. Ewes were weighed and body condition scored on two consecutive days at the beginning (d 0 and 1) and end (lambing) and once every 14 d during the treatment period. Supplement intake was adjusted for increases or decreases in body weight at every weigh day. Once ewes had lambed, the ewes and lambs were maintained in a common group on a lactation ration until weaning.

Lambs. Lambs were weighed and tagged within 24 h of birth, as well as gender, lambing difficulty, and lamb vigor recorded. Milk production was evaluated (Benson et al., 1999) utilizing ewes bearing singletons (LOW: n = 15 and MED and CON: n = 16) at an average 23 d of age. Lambs were removed from their ewes for three hours. After the three hour withdrawl, lambs were allowed to suckle until they quit suckling. Once done suckling, lambs were removed from the ewes for another three hours. Lambs were then weighed prior to being allowed to suckle again. Then lambs were allowed to suckle until they were done suckling. Once done suckling the lambs were weighed.

Lambs were weaned at an average of 69 ± 5 d of age. At weaning all lambs were placed into a common pen for a period of 20 d to acclimate to a feedlot diet. Following adaptation, wethers were allotted randomly by maternal dietary treatment and blocked by weight into a heavy or light weight pen per treatment (2 pens/treatment and 6 pens total). Wethers were weighed on two consecutive days at the beginning (d 0 and 1) and end of the feedlot period (d 109 and 110 and d 143 and 144). The d 109 and 110 wethers included all wethers meeting or exceeding at least 69 kg BW and the d 143 and 144 wethers included the remaining wethers. Once every 28 d during the feedlot period wethers were weighed. Wethers were fed ad libitum (85% corn, 15% commercial market lamb pellet; Table 2) via bulk feeders and had access to fresh water. At the end of the feedlot period, lambs were transported to Iowa Lamb Corporation in Hawarden, IA or the Department of Animal Science Meat Lab at North Dakota State University in Fargo, ND for harvest and carcass data collection.

Statistical analysis. Ewe performance, lamb weigh-suckle-weigh, and wether feedlot performance and carcass characteristics were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). The model included the main effects of treatment, ewe pen, and the interaction between the two. If the interaction was found to be clearly not significant ($P > 0.30$), it was removed from the model. The data are presented as least squares means (LSmeans) ± SEM. Significance was set at $P \leq 0.05$ and tendencies at $P \leq 0.10$.

RESULTS AND DISCUSSION

Ewe performance. Initial BW, initial BCS, and final BW pre-partum were not different ($P \geq 0.26$, Table 3) among dietary treatments. This was expected due to the randomized allotment. However, Swanson et al. (2008) observed a reduction in final post-partum BW of ewes fed a restricted diet of 60% of NRC (1985) energy requirements during the last 50 day of gestation compared with ewes fed 100 or 140% of energy requirements. Similar to the results observed by Swanson et al. (2008), Ford et al. (2007) observed an increase in pre-partum BW of ewes fed at 100% of NRC (1985) energy requirements for the early gestational ewe compared with those ewes restricted to 50% of requirements for the early gestational ewe during early to mid-gestation. Change in body condition score during the last 50 days of gestation was not affected ($P = 0.59$) by maternal MP supplementation. Although BW was not affected by dietary treatment, ewes maintained on the CON and MED diets had a greater ($P < 0.001$) increase in BW compared with the ewes maintained on the LOW diet. Stalker et al. (2006) observed similar results with cows supplemented during gestation maintaining BW compared with cows receiving no supplement during gestation. Cows supplemented with CP during the last trimester of gestation maintained BW and BCS compared with cows not supplemented (Martin et al., 2007). The reduction in BW in the LOW ewes may indicate that those ewes had to utilize body reserves to maintain pregnancy on a restricted diet and therefore lost more weight during gestation due to the lack of nutrients being fed. There were no difference ($P = 0.35$) in BW change at lambing because of maternal MP supplementation. Cows restricted to 81% of CP requirements had decreased BW and BCS within 24 h post-calving compared with cows fed 141% of CP requirements (Anthony et al., 1986). The final pre-partum BCS tended ($P = 0.09$) to be increased in the MED ewes compared with the LOW ewes. At lambing, the LOW and MED ewes had reduced ($P = 0.001$) BCS compared with CON ewes. The LOW and MED ewes had a greater ($P = 0.007$) reduction in change in BCS compared with the CON ewes. This would be explained by the reduced BCS at lambing for the restricted (LOW and MED) ewes. Increased BCS change in the restricted (LOW and MED) ewes may be indicative of using body reserves to maintain pregnancy during the last 50 days of gestation. The LOW ewes gained less weight and had increased loss in body condition during the last 50 days of gestation, which suggests that those ewes may have mobilized more body reserves to maintain pregnancy.

Offspring. Milk production and lamb weaning weight was not affected ($P \geq 0.65$, Table 4) by

ewe dietary treatment. Calves from cows supplemented with CP during gestation had increased weaning weights and ADG from birth to weaning compared with calves from non-supplemented cows (Stalker et al., 2006). There was a maternal dietary treatment by birth type interaction for birth weight ($P < 0.001$). As litter size increased the CON ewes gave birth to heavier lambs than those ewes fed the MED diet. In contrast, birth weights of lambs from ewes maintained on 60 and 140% of NRC (1985) energy requirements for the early gestational ewe were reduced compared with lambs from ewes maintained on 100% of NRC energy requirements for the early gestational ewe (Swanson et al., 2008). However, birth weights of calves born to cows supplemented with CP during gestation were not different compared with calves born to non-supplemented cows (Anthony et al., 1986; Stalker et al., 2006; Martin et al., 2007). Heifers from cows receiving CP supplementation during the last trimester tended to have increased weaning weights and 205 d adjusted weaning weights (Martin et al., 2007). The increased birth weight of lambs born to ewes on the LOW diet may also indicate a mobilization of body reserves or the ewes becoming more efficient in partitioning nutrients between themselves and the fetus during the last 50 days of gestation to maintain pregnancy.

Maternal dietary treatment did not affect ($P \geq 0.13$, Table 4) initial and final BW, ADG, feed efficiency, carcass weight, dressing percentage, LM area, back fat thickness, body wall thickness, leg score, conformation score, flank streaking, quality grade, and yield grade of wethers during the finishing phase. Back fat thickness measured via real-time ultrasonography was increased in lambs from ewes restricted to 50% of NRC (1985) energy requirements for the early gestational ewe compared with lambs from ewes maintained on 100%

Table 1. Ingredients and nutrient composition of diets fed to ewes from d 100 of gestation until lambing

	Diet[1]		
Item	LOW	MED	CON
Ingredient, %			
Corn	18.50	15.00	5.00
Dried Distiller's Grains	7.00	20.00	30.00
Soyhulls	9.50	—	—
Trace Mineral	0.49	0.49	0.49
Fescue Hay	64.51	64.51	64.51
Nutrient Composition			
DM, %	88.75	89.34	89.68
CP, % of DM	13.16	20.21	25.13
NDF, % of DM	31.03	30.73	39.79
ADF, % of DM	15.69	7.45	10.49

[1]Maternal dietary treatment: LOW: 60% of CON, MED: 80% of CON, and CON: 100% of the metabolizable protein requirement met during the last 50 days of gestation.

Table 2. Ingredient and nutrient composition of diets fed to wethers[1] during the feedlot phase

Item	Ingredient
Ingredient, %	
Whole Corn	85.0
Commercial Market Lamb Pellet[2]	15.0
Nutrient Composition	
DM	89.06
NDF, % of DM	15.54
ADF, % of DM	3.76
CP, % of DM	15.19

[1]Wethers born to ewes fed: LOW: 60% of CON, MED: 80% of CON, and CON: 100% of the metabolizable protein requirement met during the last 50 days of gestation.

[2]Commercial Market Lamb Pellet contained: 200 g/ton Chlortetracycline; 38.0% CP; 3.75-4.75% Ca; 0.6% P; 3.0-4.0% salt; 1.2 ppm Se; 24,000 IU/lb Vitamin A; 2,400 IU/lb Vitamin D; and 95 IU/lb Vitamin E.

Table 3. Effects of metabolizable protein level during the last 50 days of gestation on ewe performance

	Maternal Dietary Treatment[1]				
Item	LOW	MED	CON	SEM[2]	*P*-Value[3]
Initial BW, lb	143	143	144	2.4	0.98
Final BW pre-partum, lb	159	162	165	2.9	0.26
Weight Change, lb					
Gestation	15[b]	20[a]	21[a]	0.9	<0.001
Lambing	-28	-25	- 26	1.3	0.35
BCS					
Initial	2.9	2.9	2.9	0.03	0.64
Final pre-partum	2.9	3.0	2.9	0.03	0.09
Lambing	2.7[b]	2.8[b]	2.9[a]	0.04	0.001
BCS change					
Gestation	-0.01	0.03	0.03	0.04	0.59
Lambing	-0.18[a]	-0.20[a]	0.02[b]	0.04	0.007

[1]Maternal dietary treatment: LOW: 60% of CON, MED: 80% CON, and CON: 100% of the metabolizable protein requirement met during the last 50 days of gestation.
[2]Greatest SEM presented (n = 99 for LOW, n = 98 for MED and CON).
[3]*P* - value for the F test of the mean.
[a,b]Means within a row that lack a common superscript differ ($P \leq 0.05$; LSmeans).

of NRC requirements for the early gestational ewe (Ford et al., 2007). Supplementation of CP during gestation to cows did not affect ADG, DMI, feed efficiency, or carcass characteristics in steer calves in the feedlot (Stalker et al., 2006). However, in the current study, wethers from ewes on the LOW treatment had increased ($P = 0.01$) DMI compared with wethers from ewe on the MED treatment. In contrast to the current study, Martin et al. (2007) observed an increase in DMI in heifers from cows supplemented with CP during late gestation and fed hay during early lactation compared with cows that were pasture grazed during early lactation. The wethers from ewes supplemented with CON tended ($P = 0.10$) to have decreased days on feed in the feedlot compared with wethers from ewes supplemented with MED. Long et al. (2010) observed that calves from restricted fed cows had increased 425 days of age and both beginning and ending finishing BW compared with calves from cows fed adequately. However, the feedlot performance did not carry over to all of the carcass characteristics. Percent boneless, closely trimmed retail cuts was increased ($P = 0.04$) in wethers from ewes maintained on the CON diet compared with wethers from ewes fed the LOW and MED diets.

IMPLICATIONS

The data from the current study suggest that ewes can be fed at 60% of MP requirements (but adequate in CP and energy) and maintain pregnancy by becoming more efficient in partitioning nutrients to the fetus and mobilizing body reserves. The lambs from the ewes fed restricted MP diets may be programmed to be more efficient in partitioning nutrients for growth as well. However, this efficiency does not carry over to the majority of carcass characteristics of the lambs.

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Table 4. Effects of metabolizable protein level fed to ewes during the last 50 days of gestation on lamb performance

	Maternal Dietary Treatment[1]				
Item	LOW	MED	CON	SEM[2]	*P*-Value[3]
Birth Weight[4], lb	9.4	8.3	9.2	0.4	0.03
3 h milk production, lb	0.3	0.3	0.3	0.1	0.65
Weaning weight, lb	39.6	38.6	38.0	3.9	0.94

[1]Maternal dietary treatment: LOW: 60% of CON, MED: 80% of CON, and CON: 100% of the metabolizable protein requirement met during the last 50 days of gestation.
[2]Greatest SEM presented.
[3]*P* - value for the F test of the mean.
[4]Maternal dietary treatment x Birth Type of $P < 0.001$.
[a,b]Means within a row that lack a common superscript differ ($P \leq 0.05$; LSmeans).

Table 5. Effects of maternal metabolizable protein supplementation on feedlot performance and carcass characteristics of wethers

	Maternal Dietary Treatment[1]				
Item	LOW	MED	CON	SEM[2]	*P* - value[3]
Initial Weight, lb	65	60	67	4	0.31
Final Weight, lb	154	150	147	3	0.17
Average Daily Gain, lb/d	0.7	0.7	0.7	0.03	0.20
Days on Feed, d	127	133	123	4	0.10
G:F, lb gain: lb DMI	0.2	0.2	0.2	0.01	0.46
Dry matter intake, lb/hd/d	3.3[a]	3.2[b]	3.3[ab]	0.04	0.01
Carcass Weight, lb	79.8	76.9	75.6	1.6	0.14
Dressing Percentage, %	51.8	51.2	51.4	0.4	0.53
LM area, in^2	2.8	2.7	2.7	0.1	0.94
Back fat thickness, in	0.3	0.3	0.3	0.02	0.27
Body wall thickness, in	1.1	1.1	1.1	0.04	0.72
Leg score[4]	12	12	12	0.2	0.83
Conformation Score[4]	12	12	12	0.2	0.64
Flank Streaking[5]	362	365	395	13.5	0.13
Quality Grade[4]	12	12	12	0.1	0.29
Yield Grade	3.3	3.5	3.2	0.3	0.54
BCTRC, %[6]	44.8[b]	44.9[b]	48.3[a]	1.1	0.04

[1]Maternal dietary treatment: LOW: 60% of CON, MED: 80% of CON, and CON: 100% of the metabolizable protein requirement met during the last 50 days of gestation.
[2]Greatest SEM presented (n = 31 for LOW, n = 33 for MED, and n = 24 for CON).
[3]*P* - value for the F test of the mean.
[4]Leg score, conformation score, and quality grade: 1 = cull to 15 = $Prime^+$.
[5]Flank streaking: 100-199 = practically devoid; 200-299 = traces; 300-399 = slight; 400-499 = small; 500-599 = modest.
[6]Percent boneless, closely trimmed, retail cuts (% BCTRC) = [49.936 – (0.0848 × 2.204 × Hot Carcass Weight, kg) – (4.376 × 0.393 × 12^{th} rib fat thickness, cm) – (3.53 × 0.393 × body wall thickness, cm) + (2.456 × 0.155 × LM area, cm^2)].
[a,b]Means within a row that lack a common superscript differ ($P \leq 0.05$; LSmeans).

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Sulfur balance in lambs fed increasing concentrations of distillers dried grains with solubles[1]

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Sulfur balance of lambs fed increasing concentrations of DDGS was evaluated. Lambs fed 60% DDGS consumed 54% more water, excreted 300% more urine, and 480% more S in urine than lambs fed no DDGS. Understanding that S excretion increased with increasing dietary S concentrations explains, in part, why S toxicity did not occur.

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SUMMARY

Feeding increased concentrations of distillers dried grains with solubles (**DDGS**) has been implicated as a cause of S toxicity in ruminants. Elucidating the mechanism by which dietary S causes polioencephalomalacia (**PEM**) is of importance to the livestock feeding industry. Our hypothesis was that lambs fed increased concentrations of DDGS will increase S excretion to avoid toxicity. The objective of this study was to evaluate the effects of increasing dietary concentration of DDGS on S balance in lambs. Distillers dried grains inclusion did not affect DMI (3.0 ± 0.15 lb/hd/d; $P = 0.25$). Sulfur intake from feed and water, as well as S excretion in feces and urine increased linearly ($P \leq 0.009$) with increasing DDGS inclusion. Sulfur balance increased linearly ($P = 0.02$) with increasing inclusion of DDGS in finishing diets. Increasing concentration of DDGS in the diet increased S intake, excretion, and H_2S concentrations but did not result in the occurrence of PEM. This research suggests that substantial amounts of S contained within DDGS are excreted by the ruminant animal.

INTRODUCTION

Feeding increased concentrations of distillers dried grains with solubles (**DDGS**) to ruminants has been avoided due to risks of S toxicity and concerns about animal performance. High S diets can cause polioencephalomalacia (**PEM**) in ruminants (Gould, 1998). Feeding 60% DDGS can cause dietary S content to exceed the maximum tolerable level (0.3% S; NRC, 2005). However, research has demonstrated that lambs fed 60% DDGS (> 0.55% S) did not develop PEM (Neville et al., 2010) and performed similar to those fed lesser concentrations of DDGS (Schauer et al., 2008). Schauer et al. (2008) and Neville et al. (2010) provide an opportunity for increased utilization of DDGS in lamb finishing rations. Our hypothesis was that lambs fed increased concentrations of DDGS would increase excretion of S to avoid toxicity. The objective of this study was to evaluate the effects of increasing dietary concentration of DDGS on S balance in lambs.

Table 1. Ingredient and nutritional composition of diets fed to lambs

Item	Diet[1]			
	0% DDGS	20% DDGS	40% DDGS	60% DDGS
Ingredient, %	DM basis			
Alfalfa Hay	15.00	15.00	15.00	15.00
Corn	81.38	61.38	41.38	21.38
DDGS[2]	0.00	20.00	40.00	60.00
Ammonium Chloride	0.5	0.5	0.5	0.5
Limestone	2.25	2.25	2.25	2.25
Lasalocid[3]	0.085	0.085	0.085	0.085
TM package[4]	0.78	0.78	0.78	0.78
Copper Sulfate	0.002	0.002	0.002	0.002
Thiamin	0.011	0.011	0.011	0.011
Nutrient composition (analyzed)				
CP, %	14.0	19.4	22.0	24.7
NDF, %	23.7	27.6	30.6	31.8
ADF, %	10.1	11.0	11.1	11.5
S, %	0.22	0.52	0.70	0.84
Ca, %	1.72	1.64	1.35	1.16
P, %	0.50	0.65	0.77	0.81
Cu, ppm	19	19	15	17
Zn, ppm	59	95	90	73
Thiamin[5], ppm	70.8	67.2	55.5	51.5

[1] Diets were balanced to meet or exceed requirements set by (NRC, 2007). Treatments based on distillers dried grains with solubles inclusion: 1) 0% DDGS, 2) 20% DDGS), 3) 40% DDGS, 4) 60%DDGS.
[2] Distillers dried grains with solubles.
[3] Lasalocid (Bovatec 68, Alpharma Inc., Fort Lee, NJ).
[4] Trace Mineral (TM) package contained: 11.7% Ca, 10.0% P, 14% salt, 0.1% K, 0.1% Mg, 20 ppm Co, 100 ppm I, 2,450 ppm Mn, 50 ppm Se, 2,700 ppm Zn, 300,000 IU/lb Vitamin A, 30,000 IU/lb Vitamin D_3, and 600 IU/lb Vitamin E.
[5] Formulated based on estimated feed intake of 3 lb/d, amount of supplemental thiamin provided, and corrected for thiamin contained in remaining feed ingredients.

PROCEDURES

All animal care and handling procedures were approved by the North Dakota State University Animal Care and Use Committee prior to the initiation of the research.

Animals and Treatments. Sixteen western white-faced wether lambs (80.9 ± 5.1 lb) were utilized in a completely random design to evaluate the effects of increasing dietary concentration of DDGS on S balance and ruminal H_2S gas concentrations in lambs. Treatments were based on increasing concentrations of DDGS in the final finishing diet and included: 1) 0% DDGS, 2) 20% DDGS, 3) 40% DDGS, and 4) 60% DDGS. Treatment diets were formulated to meet or exceed CP and Cu requirements; NE was formulated for a lamb gaining 0.88 lb/d (NRC, 2007; Table 1). The dietary treatments were formulated to provide minimum Ca to P ratio of 1.5:1, and ammonium chloride (0.5%, DM basis) was added to all diets to aid in the prevention of urinary calculi. Thiamin was included in all diets at a concentration which would provide 150 mg/hd daily based on 3.0 lb estimated DMI.

Sulfur Balance. Lambs were adapted to stainless steal metabolism crates for 10 d prior to a 10 d collection period. Following adaptation, lambs were fitted with fecal collection bags. Urine was collected in plastic buckets. Feed intake was recorded daily, with daily adjustments made to target ad libitum intake (10% feed remaining daily). Feed refusals were weighed and sub-sampled daily. Water intake was calculated by subtracting any unconsumed water from water offered. Daily water samples were collected and analyzed (Stearns DHIA, Sauk Centre, MN) for sulfate (93 mg/L). Feed, ort, and fecal samples were dried using a forced-air oven (55°C; The Grieve Corporation, Round Lake, IL) for 48 h. Dried samples were ground using a Wiley Mill (Arthur H. Thomas Co., Philadelphia, PA) to pass a 2 mm screen. Samples were composited within lamb across the 10 d collection period. Samples were analyzed for S by a commercial laboratory (Midwest Laboratories, Omaha, NE).

RESULTS AND DISCUSSION

Sulfur Balance. In our study, level of dietary DDGS inclusion did not affect DMI (3.0 ± 0.15 lb/hd daily; $P = 0.25$; Table 2). Sulfur intake from feed and water, as well as S excretion in feces and urine increased linearly ($P \leq 0.009$) with increasing DDGS in the diet. Lambs fed 60% DDGS had water intakes 54% greater than those fed no

Table 2. Intake, excretion, and sulfur balance of lambs fed increasing concentrations of distillers dried grains with solubles

	Treatment[2]						*P*-Value[4]	
Item[1]	0% DDGS	20% DDGS	40% DDGS	60% DDGS	SEM[3]	*P*-value	Linear	Quadratic
Intake								
Feed, kg	1.3	1.5	1.4	1.3	0.07	0.25	0.68	0.06
S, mg	2,487.5	6,076.2	7,429.4	9,029.6	816.6	<0.001	<0.001	0.25
Water, L	3.1	3.5	3.7	4.8	0.28	0.006	<0.001	0.31
S, mg	94.8	109.4	115.7	148.9	8.7	0.006	0.001	0.31
Total S, mg	2,582.4	6,185.6	7,545.1	9,178.4	815.8	<0.001	<0.001	0.25
Excretion								
Fecal, kg	0.20	0.23	0.27	0.25	0.02	0.17	0.06	0.33
S, mg	761.4	947.6	1112.1	1130.5	90.6	0.05	0.009	0.37
Urine, L	0.59	0.85	1.1	2.4	0.3	0.008	0.002	0.12
S, mg	674.9	2,370.8	3,236.0	3,945.1	268.8	<0.001	<0.001	0.09
Total S, mg	1,436.3	3,318.4	4,348.0	5,075.6	344.5	<0.001	<0.001	0.12
Sulfur Balance, mg	1,146.1	2,867.2	3,197.1	4,102.8	568.0	0.02	0.004	0.49

[1] 1 kg = 2.205 lbs
[2] DDGS = Distillers dried grains with solubles.
[3] n = 4.
[4] *P*-value for linear and quadratic effects of increasing concentration of DDGS in diet.

DDGS ($P < 0.01$). Increased water intake resulted in an increase of 300% in urine volume and a 480% increase in urine S excretion ($P < 0.01$) compared to lambs fed no DDGS. Given the water intake and urine output data, ad libitum access to low sulfate water may be key to preventing S toxicity when high amounts of DDGS are fed. Sulfur balance increased linearly ($P = 0.004$) with increasing inclusion of DDGS in finishing diets. However, true S balance is not reported as the total volume of eructated H_2S, as well as S accumulation in wool and muscle were not measured. It is likely that substantial amounts of S were excreted via rumen gases through eructation. Therefore, further research is needed to quantify S excretion via H_2S gas and eructation.

IMPLICATIONS

Increasing concentration of DDGS in the diet increased S intake and excretion but did not result in the occurrence of PEM. Understanding that S excretion increased with increasing dietary S concentrations explains, in part, why S toxicity did not occur. The present study along with previous research at our institution has demonstrated that feeding up to 60% dietary DDGS concentrations is possible without affecting lamb health or performance if free access to low sulfate water is provided.

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Effects of maternal diet on expression of gap junctional connexins in fetal ovaries in sheep[1]

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SUMMARY

Our objective was to determine if maternal diet impacts expression of connexin (Cx) 26, 37 and 43 in fetal ovaries. Sheep were fed a maintenance (M) diet with adequate (A) selenium (Se) or high (H) Se levels from 21 days before breeding to day 135 of pregnancy. From day 50 to 135 of pregnancy (tissue collection day), a portion of the ewes from ASe and HSe groups was fed restricted (R; 60% of M) diet. Sections of fetal ovaries were immunostained for the presence of Cx26, Cx37 and Cx43 followed by image analysis. All four connexins were detected, but the distribution pattern differed. Connexin 26 was immunolocalized in the oocytes from primordial, primary, secondary and antral follicles, in granulosa and theca layers of secondary and antral follicles, stroma and blood vessels; Cx37 was expressed on the borders between oocyte and granulosa/cumulus cells of primordial, primary, secondary and antral follicles, and in endothelium; and Cx43 was expressed on cellular borders in granulosa and theca layers, and between oocyte and granulosa/cumulus cells of primordial, primary, secondary and antral follicles. Connexin 26 expression in antral follicles was decreased by R diet. The expression of Cx43 in granulosa cells of primary and granulosa and theca layers of antral follicles was increased by HSe in M diet. Thus, maternal diet affected Cx26 and Cx43 expression, and connexins may be differentially involved in regulation of fetal ovarian function in sheep, which emphasizes the importance of maternal diet in fetal growth and development.

INTRODUCTION

Fetal ovaries represent a type of tissue that expresses high tissue growth and differentiation, which are controlled by factors of fetal and maternal origin (Juengel et al. 2002, Sawyer et al. 2002, Van der Hurk & Zhao 2005; Grazul-Bilska et al. 2009). In fact, numerous factors of fetal and maternal origin, including nutrition, FSH, LH, estrogens, activin, c-kit with its ligand stem cell factor, enzymes controlling steroidogenesis, growth differentiation factor 9, epidermal growth factor and many others, may affect the

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growth, development, and physiology of the fetal and postnatal life of mammals (Borwick et al. 1997, McNatty et al. 2000, Wright et al. 2002, Pepe et al. 2006, Wu et al. 2006, Fowler et al. 2008). Additionally, our previous study demonstrated that maternal diet affected fetal body and ovarian weight, and ovarian cell proliferation in sheep (Grazul-Bilska et al. 2009).

Regulation of organ growth and function can be mediated by contact-independent and contact-dependent mechanisms (Grazul-Bilska et al. 1997). Contact-dependent cellular interactions are mediated by gap junctional pathways which facilitate and regulate the exchange of regulatory molecules (Grazul Bilska et al. 1997). Gap junctions have a hexameric structure known as a connexon that is composed of specific gap junctional proteins termed connexins (Holder et al. 1993, Grazul-Bilska et al. 1997, Kidder & Mhawi 2002, Sohl & Willecke 2003 2004, Wei et al. 2004). It has been well established that connexins not only form gap junctional channels, but also have additional functions including signal transduction, secretory function, paracrine signaling and control of growth and development of organs and tissues in physiological and pathological conditions (Moorby & Patel 2001, Serre-Beinier et al. 2002, Ebihara 2003; Goodenough & Paul 2003, Gittens et al. 2005, Borowczyk et al. 2007).

Gap junctions and/or gap junctional proteins or mRNA are expressed in the adult ovaries of several species (Grazul-Bilska et al. 1997, 1998, Johnson et al. 1999, Nuttinck *et al.* 2000, Metlon et al. 2001, Wright et al. 2001, Borowczyk et al. 2006a,b, Gershon et al. 2008). However, for fetal ovaries, expression of mRNA for several connexins and Cx43 protein has been only demonstrated in mice (Juneja 2003, Pérez-Armendariz et al. 2003). In ovaries, connexins exhibit a unique pattern of expression that can be metabolically, hormonally or developmentally regulated (Grazul-Bilska et al. 1997, Johnson et al. 1999, Gershon et al. 2008).

We hypothesized that Cx26, Cx37 and Cx43 proteins are expressed in fetal ovaries, and that expression of these connexins will be affected by maternal diet. Therefore, the objective of this experiment was to immunolocalize Cx26, Cx37 and Cx43 in ovarian compartments, and to determine if maternal plane of nutrition and Se level in the diet impacts expression of connexin proteins in fetal ovaries in sheep.

PROCEDURES

Animals and Treatments. The Institutional Animal Care and Use Committee at NDSU approved all animal procedures in this study. Animal feeding, maintenance and management are described in detail by Grazul-Bilska et al. (2009). Briefly, following breeding, pen-fed a basal diet (2.04 kg/ewe daily) ewes were assigned randomly to either an adequate (A) or high (H) dietary Se treatment. In addition, ewes were fed 100 g/day of a control pellet that contained 0.3 ppm ASe or a high-Se pellet balanced to contain 47.5 ppm Se provided as Se-enriched yeast (Sel-Plex, Alltech, Nicholasville, KY). This approach provided 6 μg/kg of ewe body weight in the ASe and 80 μg/kg of ewe body weight in the HSe treatments, respectively, The ASe and HSe pellets were formulated using similar ingredients to maintain similar concentrations of metabolizable energy (ME), crude protein, acid detergent fiber, neutral detergent fiber, Ca, and P. The approach by which dietary Se was supplemented to pregnant, primigravid ewes has been used previously by our laboratory (Carlson et al. 2008, Grazul-Bilska et al. 2009).

On day 50 of gestation, ewes within each Se treatment were stratified by average breeding date and assigned to distinct planes of nutrition treatments. Ewes were offered diets that were balanced to meet either 100% [maintenance (M)] or 60% [restricted (R)] of predicted ME requirements of pregnant ewe lambs (NRC 1985). The dietary restriction treatment was accomplished by reducing intake of restricted ewes to 60% of control treatment and therefore represents a global nutrient reduction. The plane of nutrition treatments were applied from day 50 to 135 of pregnancy, which resulted in 4 distinct treatment combinations designated by the following; MASe (n=8 ewes), MHSe (n=8), RASe (n=10) and RHSe (n=6). Only ewes with female singleton fetuses were included in this study.

Tissue Collection and Immunohistochemistry. On day 135 of the pregnancy, fetal ovaries were collected. One ovary was fixed in Carnoy's solution and the other ovary in 10% formalin solution, and after dehydration, ovaries were embedded in paraffin. Ovaries (n = 6-10/treatment) were sectioned (one section/ovary along the longitudinal axis) at 5 µm and mounted onto a glass slide.

Detection of connexins in Carnoy's fixed tissues was performed as previously described (Grazul-Bilska et al. 1998, Borowczyk et al. 2006a,b). Briefly, ovarian tissue sections were deparaffinized, rehydrated, and incubated with 3% H_2O_2 in methanol to eliminate endogenous peroxidase activity. Then, sections were rinsed several times in PBS containing Triton-X100 (0.3%, vol/vol) and treated for 20 min with PBS containing normal horse serum (3%, vol/vol; ABC kit, Vector Laboratories, Burlingame, CA) to block nonspecific binding of antibodies. Sections were incubated overnight at 4°C in PBS containing a primary rabbit polyclonal antibody against Cx26 (1:50; Zymed Laboratories Inc., San Francisco, CA), Cx37 (1:50; Alpha Diagnostics, San Antonio, TX) or Cx43 (1:500; as described by Grazul-Bilska et al. 1998). Primary antibody was detected using biotin-labeled anti-rabbit secondary antibody and the ABC method (Vector Laboratories). For color development of Cx26, Cx37 and Cx43, SG substrate was used as described before (Grazul-Bilska et al. 1998). For controls, the primary antibody was replaced with rabbit serum.

Image analysis. For all ovaries, images of stained sections were taken for each of the four types of follicles (e.g., primordial, primary, secondary and antral), and for stromal tissue within the medulla and hilus not containing follicles (total 5-15 images/ovary). The images were then used for quantitative image analysis using the Image Pro-plus software (Media Cybernetics Inc., Silver Spring, MD) to determine the proportion (%) of area stained positively for a specific connexin out of the total area of granulosa or theca layer. Image analysis was performed for Cx26 and Cx43 in primary, secondary and/or antral follicles. However, quantification of Cx37 expression using image analysis was not performed due to the heterogeneous distribution within the ovary. The number of primordial, primary, secondary and antral follicles analyzed for each connexin expression using image analysis is presented in Table 2.

Statistical Analyses. Data were analyzed as a completely randomized design with a 2 x 2 factorial arrangement of treatments using PROC GLM (SAS Inst. Inc. Cary, NC 2010). The model contained effects for plane of nutrition (M and R), level of Se (ASe and HSe), and the plane of nutrition x Se interactions. When the F-test was significant ($P < 0.05$), differences among means were separated by using the least square means procedure (Kirk 1982). Means were considered different when $P < 0.05$ unless otherwise stated. Data are expressed as mean ± SEM.

RESULTS

All three connexins were detected in fetal ovaries, but the pattern of localization of these

Table 1. Number of follicles used for image analysis of Cx26 and Cx43 expression

Follicle type	Total number of follicles analyzed		Number of follicles analyzed per nutrition group
	Cx26	Cx43	
Primary	0 (no positive staining)	33	6-11
Secondary	126	121	19-43
Antral	108	183	12-97

Table 2. Localization of Cx26, Cx37 and Cx43 in ovine fetal ovaries

Ovarian compartment	Protein
Primordial follicles	Cx37 and Cx43
Primary follicles	Cx37 and Cx43
Secondary follicles	Cx26, Cx37 and Cx43
Antral follicles	Cx26 and Cx43
Blood vessels	Cx26 and Cx37
Stromal tissues	Cx26

connexins differed (Fig. 1-3; Table 1). Connexin 26 was localized on the cellular borders and/or in the cytoplasm of cells in theca layer of secondary and antral follicles, in stromal tissues and blood vessels, and also was detected in cumulus cells of approximately 10% of antral follicles (Fig. 1). Connexin 37 was expressed as linear and punctate staining on the borders between oocyte and granulosa/ cumulus cells of primordial, primary, secondary and antral follicles, and as punctate and cytoplasmic staining in endothelium (Fig. 2). Connexin 43 was expressed as punctate staining on the cellular borders and cytoplasm in the granulosa layer of primordial, primary, secondary and antral follicles, in the theca layer of antral follicles, and on borders between the oocyte and granulosa/cumulus cells (Fig. 3).

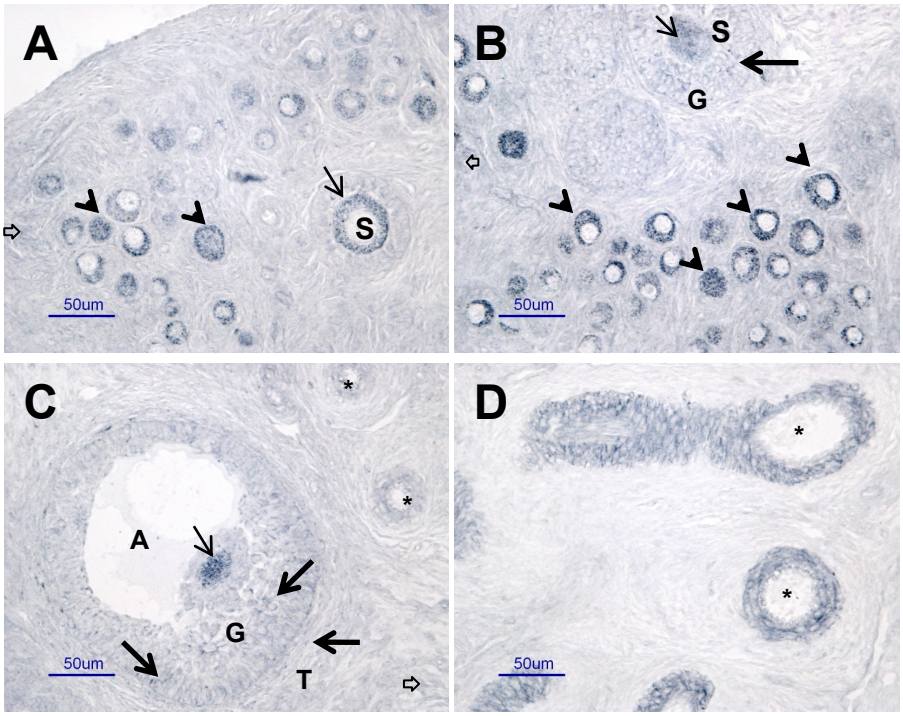

Figure 1. Representative micrograph of Cx26 immunolocalization in ovine fetal ovaries. Arrowheads indicate positive staining (dark color) in oocytes of primordial and primary follicles (A, B); small arrows indicate positive staining in oocytes of secondary (S) and antral (An) follicles (A, B, C), large arrows indicate positive staining in granulosa (G) cells of S and An follicles (B, C), and theca (T) cells of An follicle (C), open arrows indicate positive staining in stromal tissues (A, B, C), and asterisks indicate blood vessels (C, D). Control staining (no primary antibody) did not show any positive staining and was similar to the images' background (data not shown). Bar = 50 μm.

Expression of Cx26 in secondary follicles was not affected by nutritional treatments, but Cx26 expression in antral follicles was decreased (P<0.01) by high level of selenium in M and R diet (Fig. 4A). Overall, Cx26 expression in granulosa cells of antral follicles was greater (P<0.0001) than in secondary follicles (0.35±0.04 vs. 0.12±0.01%).

Expression of Cx43 in the granulosa layer of primary follicles was greater (P<0.05) in the group fed M diet with HSe than in groups fed M diet with ASe or R diet with HSe (Fig. 4B). For secondary follicles, Cx43 expression was similar in all treatment groups (data not shown). For antral follicles, Cx43 expression in granulosa and theca layers was greater (P<0.01) in the group fed M diet with HSe than in any other treatment group (Fig. 4C). Overall, Cx43 expression was greater (P<0.001) in granulosa than in the theca layer of antral follicles (1.5±0.1 vs 0.6±0.05%), and expression of Cx43 in granulosa cells was greater (P<0.001) in antral than in secondary or primary follicles (1.5±0.1 vs 0.6±0.05 or 0.5±0.06%, respectively).

DISCUSSION

The results of this experiment show that Cx26, Cx37 and Cx43 are expressed in fetal ovaries and that distribution of these connexin within ovarian compartments differs. In addition, maternal dietary restrictions and/or level of Se in the diet differentially affected Cx26 and Cx43 expression depending on fetal ovarian compartment. This suggests that maternal plane of nutrition and Se level in the maternal diet may be involved in the control of gap junction expression. These observations are novel, since very limited information is available concerning the expression of connexin proteins in fetal ovaries and regulation of their expression. In addition, these data emphasize that maternal diet may have the major impact on normal fetal ovarian growth and development, which

is a central concept of fetal/ development programming also known as developmental origins of health and disease (Nathanielsz 2006, Barker 2007).

The pattern of Cx26 localization in the thecal layer of secondary and antral follicles, in stromal tissues and blood vessels in fetal ovaries resembled the pattern observed in adult ovine ovaries (Grazul-Bilska et al. 1998). However, in contrast to adult ovine and bovine ovaries, in ovine fetal ovaries Cx26 was not expressed in surface epithelium, in the oocytes of primordial follicles, or in the granulosa layer of antral follicles (Grazul-Bilska et al. 1998, Johnson et al. 1999). Moreover, Cx26 seems to be developmentally regulated in fetal and adult ovaries since Cx26 expression was the greatest in antral follicles in this study, and in large antral follicles in adult cows (Johnson et al. 1999). The presence of Cx26 in the thecal layer of secondary and antral follicles stromal tissue and blood vessels suggests its role in the regulation of follicular development, and stromal and blood vessel function in fetal ovaries. In addition, Cx26 is likely involved in maintenance of oocyte health, since decreased Cx26 expression in cumulus oocyte complex was associated with a decreased quality of oocytes in the diabetic mouse model (Ratchford et al. 2008). However, in the present study, Cx26 was expressed in cumulus cells only in a small portion of the antral follicles present in fetal ovaries. Therefore, additional studies should be undertaken to determine what role Cx26 may have in maintenance of oocyte health in fetal ovaries in sheep.

In the present study, Cx37 protein was uniformly and consistently distributed on oocyte-cumulus/granulosa borders from primordial to antral follicles and in endothelium. Similarly, in adult ovine ovaries, Cx37 protein was detected on the oocyte/ cumulus border and in endothelial cells (Borowczyk et al. 2006b). In addition, Cx37 was present in granulosa cells of adult ovine and bovine antral follicles and murine oocytes (Nuttinck et al. 2000, Teilmann 2005, Borowczyk et al. 2006b, Ratchford et al. 2008). For the mouse, it has been clearly demonstrated that Cx37 is critical for normal oogenesis/ folliculogenesis (Simon et al. 1997; Gittens & Kidder 2005). Localization of Cx37 on the border between oocyte and cumulus/granulosa cells in all follicular stages in fetal ovaries in the present study, suggests that Cx37 may be as important for ovine oocyte/follicle development as for mice.

Connexin 26 and Cx37, but not Cx43, were expressed in blood vessels in fetal ovaries in this study. In fact, a dense network of blood vessels in the medulla and hilus and a less dense network in the cortex is present in ovine fetal ovaries (Grazul-Bilska et al. 2009). It has been demonstrated that several connexins including Cx37, Cx40, Cx43 and Cx45 are involved in the regulation of vascular function and angiogenesis in several organs in adults (Haefliger et al. 2004, Schmidt et al. 2008, Johnstone et al. 2009). It seems that type of connexin expressed in blood vessels in fetal ovaries

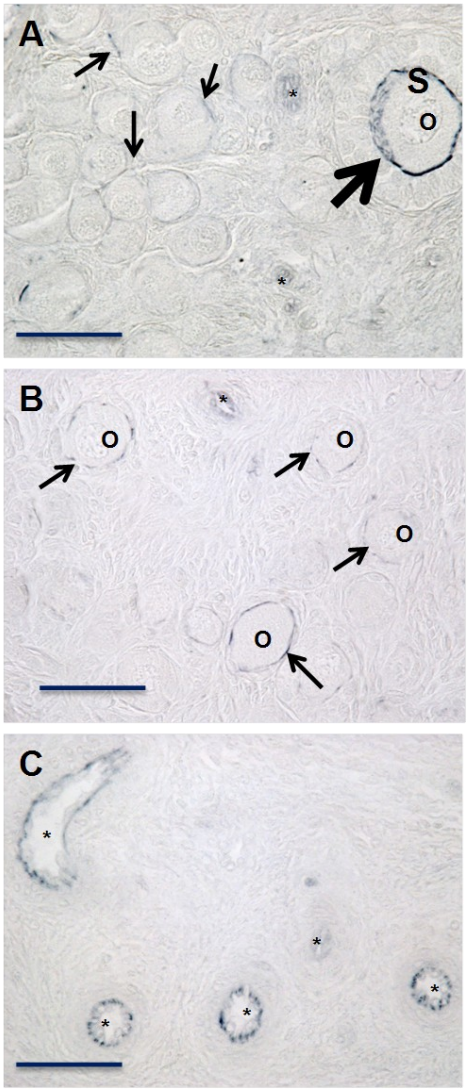

Figure 2. Representative micrograph of Cx37 immunolocalization in ovine fetal ovaries. Small arrows indicate positive Cx37 staining (dark color) on the border between oocyte (O) and granulosa (G) layer of primordial and primary follicles (A, B). Large arrow indicates positive Cx37 staining on the border between O and G layer of secondary (S) follicle (A). Asterisks indicate positive staining in blood vessels (C). Control staining (no primary antibody) did not show any positive staining and was similar to the images' background (data not shown). Bar = 50 μm.

differs from adult organs, since in fetal ovaries we detected the presence of Cx26 but not Cx43. This difference is likely due to the early developmental stage of fetal ovaries. Therefore in fetal ovaries, Cx26 and Cx37 are likely involved in regulation of blood vessel function and angiogenesis.

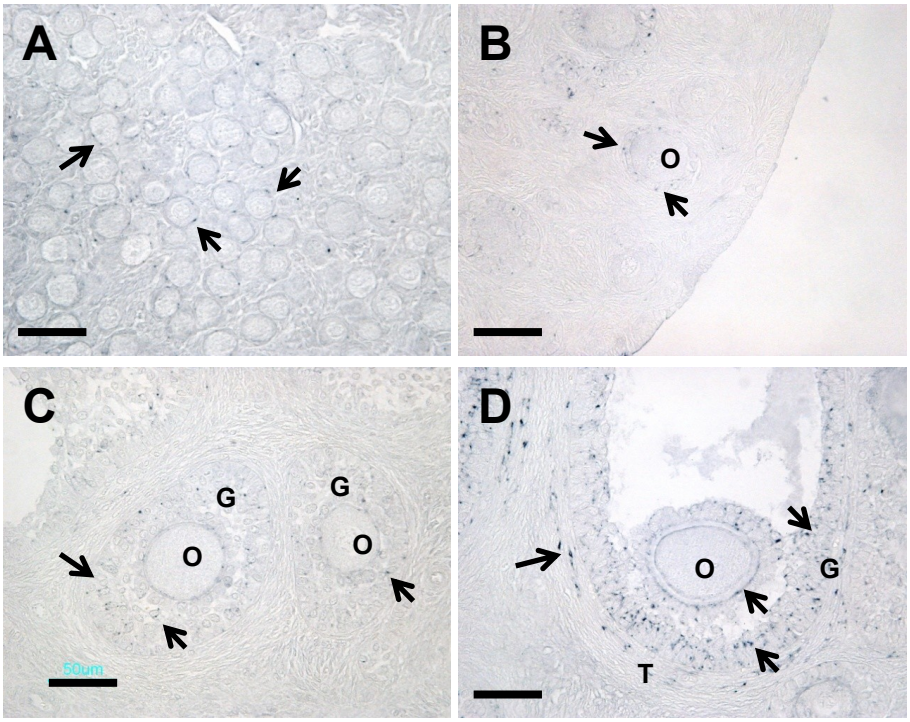

Figure 3. Representative micrograph of Cx43 immunolocalization (dark color) in primordial (A), primary (B), secondary (C) and antral (D) follicles in ovine fetal ovaries. Arrows indicate positive staining in flat granulosa (G) layer (A); in G layer of primary and secondary follicle (B, C); in G and theca (T) layers, and on the border between oocyte and G/cumulus cells in antral follicle (D). Bar = 50 μm.

Connexin 43 was exclusively expressed in granulosa and theca layer in all follicle types, and its expression level increased from the primary to antral stage of follicular development in the present study. Similarly, Cx43 was detected during fetal ovarian development in the mouse (Pérez-Armendariz et al. 2003). In addition, a similar spatio-temporal pattern of Cx43 expression was observed for adult ovaries in several species (Grazul-Bilska et al. 1998, Johnson et al. 1999, Nuttinck et al. 2000, Teilmann 2005). Thus, the Cx43 expression pattern suggests that Cx43 is involved in the regulation of folliculogenesis in ovine fetal ovaries. In addition, it has been clearly demonstrated that level of Cx43 expression in cumulus cells may serve as a marker of oocyte quality in humans (Wang et al. 2009) and that Cx43 is directly associated with steroidogenesis in the ovine ovary (Borowczyk et al. 2007). The importance of Cx43 for follicular development was emphasized in a mouse knockout model showing a lack of development beyond primary follicles (Juneja et al. 1999, Gershon et al. 2008). Thus, it is reasonable to postulate that in fetal ovaries similar to adult ovaries, Cx43 plays a major regulatory role during folliculogenesis.

In this study, Cx26 expression in antral follicles was decreased by HSe in M and R diet, and Cx43 expression in primary and antral follicles was increased by HSe level in M diet but not by maternal dietary restrictions. These differences may be due to a different localization of each connexin, to the specific stage of follicular development, and possibly to the different functions of Cx26 vs. Cx43 in fetal ovaries. Differential effects of maternal diet on fetal ovarian weight, delayed ovarian growth and development, and altered ovarian cell proliferation and apoptosis have been reported for sheep (Borwick et al. 1997, Rae et al. 2001, Osgerby et al. 2002, Da Silva et al. 2002, 2003, Grazul-Bilska et al. 2009). In addition, it has been demonstrated that high Se level in diet affects increased Cx43 dephosphorylation in rat heart which may contribute to arrhythmogenesis (Rakotovao et al. 2005). Recently, we clearly demonstrated that both maternal dietary restrictions and/or HSe level decreased cellular proliferation in primordial, secondary and/or antral follicles, stromal tissues and blood vessels in fetal ovaries (Grazul-Bilska et al. 2009). Interestingly, the enhanced Cx43 expression observed in the present study in primary and antral follicles in the group fed M diet with HSe corresponds to decreased cellular proliferation in the same compartments of fetal ovaries from the same nutrition-

treatment group (Grazul-Bilska et al. 2009). Since Cx43 is recognized as an important regulator of cell and tissue growth (Grazul-Bilska et al. 1997, Moorby & Patel 2001), we hypothesize that increased expression of Cx43 may be a part of a compensatory mechanism preventing an additional decrease of cell proliferation and maintaining follicle integrity in fetal ovarian follicles when cell proliferation is reduced by HSe in the diet. In fact, it has been demonstrated that Se is involved in regulation of several cellular functions such as cell proliferation and angiogenesis in many organs (Salbe et al. 1990, Jiang et al. 1999, Zeng 2002, Yeh et al. 2006, Carlson et al. 2008, Zeng & Combs 2008). This study suggests that Se is involved in the regulation of Cx43 expression. However, additional studies should be undertaken to determine the mechanism of the effects of the level of energy and Se in maternal diet on regulation of gap junction expression and function in fetal tissues.

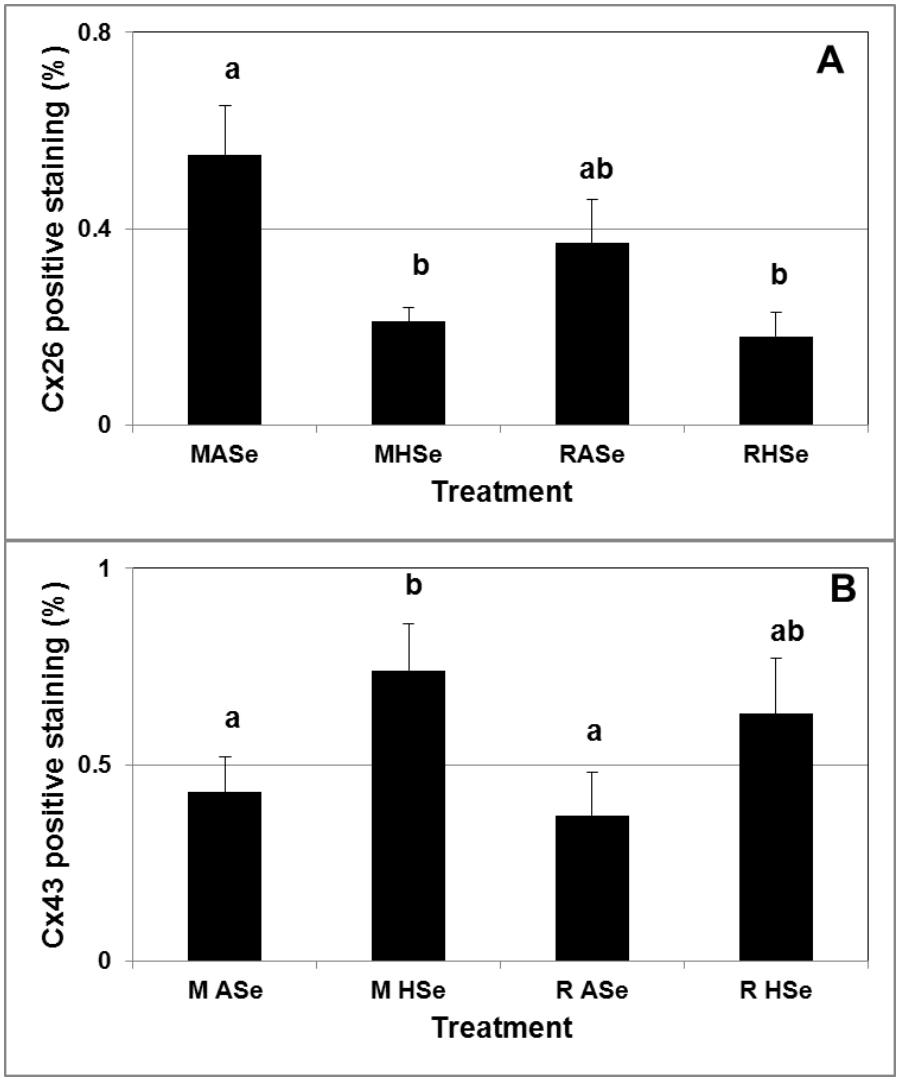

Figure. 4. Effects of maternal nutrition on expression of Cx26 in antral follicles (A) and Cx43 in primary (B) and antral follicles (C). $^{a,b}P < 0.01$ for Cx26 and 0.04 for Cx43; means ± SEM with different superscripts differ within follicle type.

In summary, Cx26, Cx37 and Cx43 are expressed in several ovarian compartments but the distribution of these connexin differed within fetal ovaries. The presence of these connexins in fetal ovaries suggests that they may play a differential role in gap junction mediated regulation of growth and function of fetal ovaries. Expression of Cx26 and Cx43 was affected by the stage of follicular development and by maternal diet; nutrient restriction decreased Cx26 expression in thecal layer of antral follicles, and high Se concentration in M diet increased Cx43 expression in granulosa cells of primary and granulosa and theca layers of antral follicles. Therefore, we postulate that Cx26 and Cx43 are involved in the regulation of folliculogenesis. Our results emphasize the importance of maternal diet in the regulation of fetal ovarian growth. The results of this study will help us begin to understand the role of maternal diet in regulation of gap junction function in fetal tissues.

Although we have demonstrated the effects of maternal diet on fetal ovarian development and suggested a role for connexins in the process, it is currently unclear if dietary supplements would affect oocyte quality and thus the reproductive function of the offspring. Therefore, more research is required to demonstrate if exposure of the fetus to specific environmental factors may affect reproductive function in the offspring (Gardner *et al.* 2008). Nevertheless, the knowledge generated

in this study will likely contribute to the concept of fetal programming/fetal origin of adult disease.

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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 ewe 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 them 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 ewes 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.
3. 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.
4. 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.