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Growth performance and carcass characteristics of conventionally raised lambs implanted with zeranol versus naturally raised lambs[1]

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The objective of this research was to elucidate the advantages of conventional lamb feeding systems in comparison to lambs raised in accordance with naturally raised guidelines. Voluntary standards released by the Agriculture Marketing Service provide feedlot operations with procedures to produce naturally raised lamb in agreement with consumer perceptions of the product. If lambs can be raised within these guidelines as cost effectively as lambs raised in a best management practice operation and sold at a premium, it could provide lamb feedlot operations with an alternative to conventional programs while increasing profitability.

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Summary

The objective of this research was to compare the growth performance and carcass characteristics between naturally raised and conventionally raised lambs. Two hundred eighty eight crossbred lambs (75 lbs.) were finished in twelve feedlot pens according to treatment over a 112 day trial. Treatments were Naturally Raised (**NR**) or Conventional (**C**). Naturally Raised lambs were fed a basal diet (80% corn, 20% concentrate) with decoquinate added for coccidiosis control, and could not be treated with antibiotics. Conventional lambs were fed a similar 80:20 basal ration, but with decoquinate, chlortetracycline and lasalocid added. Conventional lambs were also implanted with 36 mg zeranol (Ralgro®, Schering-Plough) on day 28, and could be treated with antibiotics as necessary. After 112 days, lambs were harvested and carcass data was collected. Conventional lambs had increased average daily gain ($P = 0.06$). Naturally Raised lambs had increased rib eye area ($P = 0.03$), decreased body wall thickness ($P = 0.05$), and increased boneless, closely trimmed retail cuts ($P = 0.05$). Conventional lambs also had increased percentage of lambs with rectal or vaginal prolapses ($P = 0.001$) and increased percent mortality ($P = 0.01$). Conventional lambs trended to be heavier at the final weight ($P = 0.07$) and have increased dry matter intake ($P = 0.09$). There were no differences in yield grade ($P = 0.25$), feed efficiency ($P = 0.47$), or quality grade ($P = 0.85$). While C lambs gained more, the viability of the zeranol is put into question by the high incidence of prolapse and mortality. Future research should evaluate graded levels of zeranol implants to discern if the incidence of prolapse and mortality can be decreased.

Introduction

The USDA Agriculture Marketing Service released voluntary standards for the production of naturally raised livestock in January of 2009. *The United States Standards for Livestock and Meat Marketing Claims, Naturally Raised Claim for Livestock and the Meat*

and Meat Products Derived From Such Livestock provided guidelines for what should be marketed as naturally raised livestock. The core of the report stated that naturally raised livestock should be raised "… without growth promotants and antibiotics and that have never been fed mammalian or avian by-products…" Niche marketing (including naturally raised or organic) has steadily grown in popularity over the last decade. Farmers and ranchers could take advantage of this growing market if the requirements were better understood, and the economic benefits were clearly demonstrated.

Naturally raised lambs must be able to compete with conventional lambs that can be both supplemented with lasalocid or chlortetracycline at levels to improve growth efficiency and be implanted with zeranol. Zeranol has been shown to increase average daily gain (**ADG**) and feed efficiency in lambs (Hustfedler et al., 1996; Nold et al., 1992; Salisbury et al., 2007). Chlortetracycline (**CTC**), an antibiotic often used as a broad-spectrum antibiotic in livestock operations, has been shown to improve ADG, feed efficiency, and survival rate (Johnson et al., 1956; Bridges et al., 1953; Kunkel et al., 1956). Lasalocid, a polyether ionophore, has also been shown to increase ADG and feed efficiency and decrease incidence of coccidia in steers (Anderson et al, 1988; Bartley et al., 1979; Berger et al., 1981) and increase ADG and improve feed efficiency in lambs (Funk et al., 1986). Naturally raised lambs must perform at the same level as conventional lambs or producers must receive a premium in order to justify naturally raised lamb production.

Procedures

Two hundred eighty eight spring born crossbred lambs (wethers and ewes) were stratified by weight and assigned randomly within stratification to one of two treatments (Naturally Raised or Conventional) in a completely randomized design to evaluate lamb growth performance and carcass characteristics under naturally raised and conventional management practices. At the start of the trial, lambs were moved to 12 feedlot pens (n = 6). Each pen represented one experimental unit and contained 24 lambs. Treatments were randomly assigned to pens. Treatments consisted of Naturally Raised and Conventional. Naturally raised (**NR**) lambs were fed the basal feedlot ration, and supplemented with decoquinate (Deccox®). Naturally raised lambs could not receive any other antibiotics. If treatment with antibiotics was necessary, an ear notch was administered to the treated lamb and it was removed from the data set. Conventional (**C**) lambs were raised using best management practices, including supplementation with decoquinate (Deccox®), lasalocid (Bovatec®), CTC, and implanted with zeranol (Ralgro®). Thirty six mg zeranol were administered via subcutaneous implant in the ear on d 28. The feedlot ration for treatment C consisted of 78.7% corn, 19.7% market lamb pellet, 1.2% Deccox®, and 0.4% CTC on a dry matter basis. The C market lamb pellet contained lasalocid and 38% crude protein. The feedlot ration for treatment NR was 80% corn and 20% market lamb pellet, which contained decoquinate and 38% crude protein. Lambs were fed ad-libitum via bulk feeders and had access to fresh water. Refusals were collected every 28 days.

Experimental Periods and Sampling Procedures. The experiment began May 15, with lambs weighed two consecutive days to determine starting body weight. Lambs were weighed once every 28 days after initial weights, with two consecutive weights taken at the end of the trial. Lambs were then shipped for harvest and carcass data collection at Iowa Lamb Corporation in Hawarden, IA. Feed samples (approximately 0.44 lb) were collected approximately once every 28 d, dried at 55°C for 48 h, ground through a Wiley mill (1-mm screen), and composited for analysis of ADF and NDF, N, and OM.

Statistical Analysis. Lamb performance data was analyzed as a completely randomized design using the MIXED procedure of SAS with replication serving as experimental unit. The contrast statements included the linear effects of management practices. Response variables included: 1) lamb growth performance; 2) carcass data; 3) incidence of rectal and vaginal prolapse; and 4) mortality.

Results and Discussion

Conventional management practices showed advantages and disadvantages in comparison to naturally raised management. Conventional lambs had increased ADG ($P = 0.06$) and subsequently increased gain ($P = 0.06$) over the 112 d trial, illustrating the growth effects of CTC, lasalocid, and zeranol displayed in previous research. The increase in gain, which averaged approximately five pounds across treatments, makes conventional management an economically viable option when compared to naturally raised lambs. The implants costs roughly one dollar per head, and the five pounds gained (assuming market price of one dollar per pound) covered the cost of implants as well as provided a four dollar increase in profit compared to naturally raised lambs (not including labor). Extrapolated to a 2000 head feedlot, conventional practices with zeranol could result in an $8,000 per year increase return. While C lambs had a trend of increased DMI ($P = 0.09$) the overall economic effects were minimal. Conventional lambs also showed a trend of increased ADG and gain during early treatment periods. The difference between treatments may have been larger had the lambs been slaughtered earlier. Lambs were slaughtered at an average weight of 159 lbs, and differences between treatments may have diminished as gain decreased in the larger C lambs late in the trial.

Most carcass characteristics were similar between treatments ($P \geq 0.25$), with the exceptions of rib eye area (**REA**), body wall thickness (**BWT**), and boneless, closely-trimmed retail cuts (**BCTRC**). Naturally Raised lambs had larger REA when compared to C lambs ($P = 0.03$). However, the numeric difference between treatment averages was less than one tenth of an inch (2.66 vs. 2.57 in^2). Naturally raised lambs also had a thicker body wall ($P = 0.05$) and greater BCTRC ($P = 0.05$). This could suggest C lambs had decreased carcass performance, but the authors feel this was influenced by the extended trial length. However, the major cause for concern in the trial was the high incidence of rectal and vaginal prolapses and mortality in the C treatment. The twelve prolapses in the C treatment were significantly more than the NR treatment, which resulted in no prolapses ($P = 0.001$). Of the twelve lambs prolapsed, some had to be treated repeatedly, and eventually four died ($P = 0.01$). The advantages of the increased gain are most likely negated by the cost of treating prolapsed lambs and mortality as a result of complications from prolapses. Zeranol has often been cited as an instigator in increased prolapses anecdotally, and a trend of increased prolapses was observed in a trial by Salisbury et al (2007).

Implications

Decreased growth performance in naturally raised lamb demands premiums be offered to lamb producers in order for natural lamb production to be an economically viable practice, despite the possibility of improved carcass characteristics. While increased prices are afforded producers who sell products in niche markets such as

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

	Diets[1]	
Item	C	NR
Ingredient	DM basis	
Whole Corn, %	78.68	80
C Market Lamb Pellet[2], %	19.77	0
NR Market Lamb Pellet[3], %	0	20
Deccox, %	1.18	0
Chlortetracycline,%	0.37	0
Nutrient composition		
CP, %	17.59	17.64
TDN, %	85.99	86.62
Ca, %	1.00	1.02
P, %	0.33	0.33

[1] Treatments abbreviations C (conventional), NR (naturally raised).
[2] Conventional Market Lamb Pellet contained: 136 g/ton lasalocid, 38% CP, 3.75-4.75% Ca, 0.6% P, 3.0-4.0% salt, 1.2ppm Se, 24,000 IU/lb Vitamin A, 2,400 IU/lb Vitamin D, and 70 IU/lb Vitamin E.
[3] Naturally raised Market Lamb Pellet contained: 65mg/lb (0.01432%) decoquinate, 38% CP, 3.75-4.75% Ca, 0.6% P, 3.0-4.0% salt, 1.2ppm Se, 24,000 IU/lb Vitamin A, 2,400 IU/lb Vitamin D, and 70 IU/lb Vitamin E.

farmers markets or directly to restaurants, large-scale feedlot operations using naturally raised management techniques require a premium for naturally raised lamb to offset the potential loss in growth performance compared to conventionally raised lambs. Alternatively, the increased performance in conventionally raised lambs, which may be attributed to zeranol implants, offers economic opportunities if a level of zeranol dosage can be found to improve growth without increasing incidences of prolapse. Future research will focus on determining if decreased levels of zeranol will produce increased gain without increased prolapses and decreased carcass quality.

Table 2. Comparison of Conventional and Naturally Raised feeding practices on feedlot lamb performance and carcass characteristics

	Treatment[1]			
Item	C	NR	SEM[2]	*P*-value[3]
Initial Wt, lbs	75	75	0.28	0.96
Final Wt, lbs	162	157	1.57	0.07
ADG, lbs/d	0.77	0.73	0.01	0.06
Intake, lbs DM/hd/d	3.60	3.47	0.05	0.09
G:F, lbs gain: lbs DMI	0.21	0.21	0.003	0.47
Gain, lbs	87	82	1.48	0.06
HCW, lbs	81.5	80.4	0.8	0.35
Leg Score[4]	11.5	11.5	0.07	0.95
Conformation score	11.5	11.6	0.06	0.5
Fat Depth, in[5]	0.33	0.31	0.01	0.25
Body Wall Thick, in	1.11	1.06	0.01	0.05
Ribeye Area, in^2	2.57	2.66	0.02	0.03
Flank Streaking[6]	351.03	356.89	5.85	0.5
Quality Grade	11.4	11.4	0.06	0.85
Yield Grade[7]	3.72	3.55	0.1	0.25
%BCTRC[8]	43.57	43.92	0.11	0.05
Lean, lbs	35.4	35.2	0.28	0.69
Dress, %	49.26	49.26	0.15	0.99
Prolapse, %	0.083	0	0.01	0.001
Mortality, %	0.028	0	0.006	0.01

[1]Treatments abbreviations C (conventional) NR (naturally raised)

[2]Standard Error of Mean; n = 6.

[3]*P*-value for F-tests of mean

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

[5]Adjusted fat depth and yield grades.

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

[7]Yield Grade = 0.4 + (10 x adjusted fat depth).

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

Literature Cited

Anderson K.L., T.G. Nagaraja, J.L. Momll, P.G. Reddy, T.B. Avery, and N.V. Anderson. 1988. Performance and ruminal changes of early-weaned calves fed lasalocid. J. Anim. Sci. 66:806-813.

Bartley E.E., E.L. Herod, R.M. Bechtle, D.A. Sapienza, B.E. Brent and A. Davidovich. 1979. Effect of monensin or lasalocid, with and without niacin or amicloral, on rumen fermentation and feed efficiency. J. Anim. Sci. 49:1066-1075.

Berger L.L., S.C. Ricke and G.C. Fahey, Jr. 1981. Comparisons of two forms and two levels of lasalocid with monensin on feedlot cattle performance. J. Anim. Sci. 53:1440-1445.

Bridges J.H., J.C. Miller, W.G. Kammlade, Jr. and H.O. Kunkel. 1953. Effects of various levels of Aureomycin in fattening lambs. J. Anim. Sci. 12:660.

Funk M.A., M.L. Galyean and T.T. Ross. 1986. Potassium and lasalocid effects on performance and digestion in lambs. J. Anim. Sci. 63:685-691.

Hufstedler, G.D., P.L. Gillman, G.E. Carstens, L.W. Greene, and N.D. Turner. 1996. Physiological and hormonal responses of lambs repeatedly implanted with zeranol and provided two levels of feed intake. J. Anim. Sci. 74:2376-2384.

Johnson W.P., R. F. Elliott and A. L. Shor. 1956. The effect of chlortetracycline on the incidence of enterotoxemia and weight gain in lambs maintained under commercial feedlot conditions. J. Anim. Sci. 15:781-787.

Kunkel H.O., L.P. Packett Jr., M Hoelscher, and J.H. Bridges. 1956. Chlortetracycline supplements in self-fed rations for lambs. J. Anim. Sci. 15:770-780.

Nold, R.A., J.A. Unruh, C.W. Spaeth, and J.E. Minton. 1992. Effect of zeranol implants in ram and wether lambs on performance traits, carcass characteristics, and subprimal cut yields and distribution. J. Anim. Sci. 70:1699-1707.

Salisbury, M.W., B.J. May, S.J. Talley, M.A. Carr, and G.R. Engdahl. 2007. Feedlot performance and carcass characteristics of feeder lambs implanted and re-implanted with zeranol. Texas J. of Ag. And Nat. Res. 20:1-9.

Wilson, L.L., H. Varela-Alvarez, M.C. Rugh, and M.L. Borger. 1972. Growth and carcass characteristics or rams, cryptorchids, wethers, and ewes subcutaneously implanted with zeranol. J. Anim. Sci. 34:336-338.

Influence of thiamin supplementation on hydrogen sulfide gas concentrations in ruminants fed high sulfur diets

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The objective of this research was to evaluate the influence of thiamin supplementation on hydrogen sulfide gas concentration and ruminal pH in lambs fed high sulfur diets. Moderate levels of thiamin supplementation seem to decrease hydrogen sulfide concentrations. Our data suggests that changes in ruminal hydrogen sulfide concentration cannot be attributed solely to ruminal pH, and are likely affected by multiple factors which interact within the ruminal environment and in the animal.

[1]Partial support for this research and distiller dried grains with solubles were provided by Poet Nutrition, Sioux Falls, SD. Disclaimer: Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of Poet Nutrition.

Summary

The objective of this study was to evaluate the effect of increasing level of thiamin supplementation on ruminal gas cap hydrogen sulfide (H_2S) concentration and pH in lambs. Twenty crossbred lambs (84.5 ± 7.0 lb) were adapted over 28 d to a finishing diet consisting of (DM basis) 60% distillers dried grains with solubles, 21.4% corn, 15% alfalfa hay, and 3.6% supplement. Treatments diets differed in the amount of supplemental thiamin supplied; diets were formulated to provide: 1) **CON** (no supplemental thiamin), 2) **LOW** (50 mg·hd^{-1}·d^{-1} thiamin), 3) **MED** (100 mg·hd^{-1}·d^{-1} thiamin), 4) **HIGH** (150 mg·hd^{-1}·d^{-1} thiamin), or 5) **HIGH+S** (150 mg·hd^{-1}·d^{-1} thiamin with dietary S increased from 0.71% to 0.87% (DM basis) with the addition of dilute sulfuric acid to DDGS). Thiamin supplementation was based on an estimated daily DMI of 3 lb·hd^{-1}·d^{-1}. Hydrogen sulfide and rumen fluid pH were collected via rumen puncture on d -6, -3, 0, 3, 7, 10, 14, 17, 21, 24, 28, and 31. No differences in H_2S concentration ($P > 0.10$) between treatments were apparent until d 10, at which point lambs fed LOW had lower H_2S concentrations than all other treatments. Lambs fed HIGH had the greatest concentrations of H_2S on d 31 (7700 ppm H_2S; $P < 0.009$). Ruminal pH for lambs fed CON and MED were not different from d 0 throughout sampling ($P > 0.18$). Ruminal pH of LOW, HIGH, and HIGH+S groups decreased ($P < 0.03$) over time. Thiamin appears to influence ruminal H_2S concentrations, although the mechanism by which this occurred remains unknown. Changes in H_2S concentration cannot be attributed solely to ruminal pH, and are likely affected by multiple factors which interact within the ruminal environment and in the animal.

Introduction

One of the challenges with use of ethanol co-products is the potential for high dietary S levels. High S diets can cause polioencephalomalacia (**PEM**) in ruminants. Inclusion of large percentages of co-product feeds, like distillers dried grains with solubles (**DDGS**), in finishing

rations has been avoided, in part, due to problems with PEM as well as concerns about optimal animal performance and carcass characteristics. Thiamin supplementation is one proposed method of reducing or preventing PEM in ruminant animals. The efficacy of thiamin supplementation in preventing PEM is likely impacted by the mechanisms by which PEM is caused (e.g. long-term thiamin deficiency or high hydrogen sulfide gas concentration). Further, the effect and dose of thiamin necessary to prevent such cases of PEM requires more investigation. Hydrogen sulfide gas, as previously mentioned has been implicated as a cause of PEM in ruminants. Both high sulfur feed (Niles et al., 2002) and water (Loneragan et al., 2005) sources can cause increases in H_2S production. Presently there is no published literature evaluating the effect of dietary thiamin concentrations on ruminal H_2S gas concentration. Therefore, our objective was to evaluate the effect of increasing level of thiamin supplementation on ruminal gas cap H_2S concentration and ruminal pH in lambs being adapted to a finishing diet containing 60% DDGS.

Procedures

Twenty western white-face wether lambs (84.5 ± 7.0 lb) were sampled during the adaptation period (receiving ration to a final finishing ration). Adaptation was accomplished by increasing the amount of concentrate on a weekly basis; adaptation diets are outlined in (Table 1). The final finishing diet was balanced to contain 60% DDGS (DM basis; Table 2). Treatments diets differed in the amount of supplemental thiamin supplied; diets were formulated to provide: 1) **CON** (no supplemental thiamin), 2) **LOW** (50 $mg \cdot hd^{-1} \cdot d^{-1}$ thiamin), 3) **MED** (100 $mg \cdot hd^{-1} \cdot d^{-1}$ thiamin), 4) **HIGH** (150 $mg \cdot hd^{-1} \cdot d^{-1}$ thiamin), or 5) **HIGH+S** (150 $mg \cdot hd^{-1} \cdot d^{-1}$ thiamin with dietary S increased from 0.71% to 0.87% (DM basis) with the addition of dilute sulfuric acid to DDGS). Thiamin supplementation was based on an estimated daily DMI of 3 $lb \cdot hd^{-1} \cdot d^{-1}$. Feed was offered daily on an ad libitum basis with refusals collected and weighed weekly.

Sampling for ruminal H_2S was conducted on 12 occasions beginning 6 d prior to initiation of treatment diets. Gas cap samples from these lambs were collected on d -6, -4, 0, 3, 7, 10, 14, 17, 21, 24, 28, and 31 of the feeding period. Hydrogen sulfide gas measured on H_2S detector tubes (GASTEC©, Kanagawa, Japan). Ruminal fluid was also collected at the same time for determination of rumen fluid pH.

Results

The influence of hydrogen sulfide gas on incidence of PEM in ruminants could be impacted by the way H_2S concentration changes during adaptation to finishing rations. In the present study, no differences in H_2S concentration between treatments ($P > 0.10$; Table 3) were apparent until d 10, at which point lambs fed LOW had lower H_2S concentrations than all other treatments. At this point in adaptation the amount of roughage included in the diet had not changed although the inclusion of DDGS had increased from 0 to 29% of dietary DM. Those lambs fed the HIGH treatment diet showed the most dramatic increases in ruminal H_2S concentration; on d 21 of adaptation dietary hay was decreased from 35 to 25% and DDGS increased from 40 to 50% of dietary DM. Over the course of the next 3 d ruminal H_2S concentration increased by over 3000 ppm, and within 7 d had increased by 4700 ppm H_2S.

While the hydrogen sulfide concentrations in our lambs did not reach the levels in steers reported by Niles et al. (2002), our peak concentrations were above those reported by Loneragan et al. (2005); both of these studies had steers with positive cases of PEM. These results indicate that the concentration of H_2S required to cause symptoms of PEM may vary depending on species.

Of further interest is the way H_2S concentration in lambs fed HIGH+S changed over adaptation. Specifically, on days 7, 14, and 21 the concentration of H_2S was greater in HIGH+S than HIGH; however, after 3 d of adaptation (d 10, 17, 24) the concentration of ruminal H_2S from HIGH+S was lower or equal to that found in HIGH fed lambs.

There are multiple factors that influence the conversion of dietary S into H_2S in the rumen

during adaptation. Among these are decreases in ruminal fluid pH, increases in the proportion of sulfur reducing bacteria, and increases in dietary S. In our study, ruminal pH did not differ among treatments (P = 0.13) at any time point (data not shown). Lambs fed CON and MED were not different from d 0 throughout sampling (P > 0.18). However, ruminal pH of LOW, HIGH, and HIGH+S groups did decrease (P < 0.03) over time. Decreases in ruminal pH may also impact incidence of PEM by other means.

Our research suggests that thiamin may influence ruminal H_2S concentrations, but we did not investigate the fate of the H_2S. Further, our data suggests that changes in ruminal hydrogen sulfide concentration cannot be attributed solely to ruminal pH, and are likely affected by multiple factors which interact within the ruminal environment and in the animal.

Table 1. Adaptation diets fed to lambs (% DM Basis)

	Arrival d -6	Step 1 d 0	Step 2 d 7	Step 3 d 14	Step 4 d 21	Step 5 d 28
Ingredient, %						
Alfalfa Hay	46.00	46.00	46.00	35.00	25.00	15.00
Corn	50.38	35.88	21.38	21.38	21.38	21.38
DDGS	0.00	14.50	29.00	40.00	50.00	60.00
Supplement[1]	3.62	3.62	3.62	3.62	3.62	3.62

[1]Supplement contained: (% of total diet DM) 0.5% ammonium chloride, 2.25% limestone, 0.085% lasalocid, 0.78% trace mineral, 0.002% copper sulfate, and were formulated to provide one of four levels of thiamin (0, 50, 100, or 150 mg·hd^{-1}·d^{-1}).

Table 2. Ingredient and nutritional composition (DM basis) of final finishing rations fed to lambs

	Treatments[1]				
Item	CON	LOW	MED	HIGH	HIGH+S
Ingredient,%					
Alfalfa Hay	15.00	15.00	15.00	15.00	15.00
Corn	21.38	21.38	21.38	21.38	21.38
DDGS	60.00	60.00	60.00	60.00	60.00
Supplement[2]	3.62	3.62	3.62	3.62	3.62
Nutrient[3]					
CP, %	23.3	23.6	23.4	22.7	23.5
ADF, %	10.8	11.0	11.6	11.6	11.3
S, %	0.76	0.69	0.75	0.71	0.87
Ca, %	1.55	1.42	1.65	1.66	1.77
P, %	0.79	0.81	0.92	0.91	0.87
Thiamin[4]	0	50	100	150	150

[1] Treatments: CON (no supplemental thiamin), LOW (50 mg·hd^{-1}·d^{-1} thiamin), MED (100 mg·hd^{-1}·d^{-1} thiamin), HIGH (150 mg·hd^{-1}·d^{-1} thiamin), and HIGH+S (150 mg·hd^{-1}·d^{-1} thiamin with 0.87% S).
[2] Supplement (% total diet): 0.5% Ammonium chloride, 2.25% limestone, 0.085% Lasalocid, 0.78% Sheep Mineral 12 (Hubbard Feeds, Mankato MN), 0.002% Copper sulfate, and either 0, 0.004, 0.007, or 0.11% thiamin mononitrate.
[3] Laboratory analysis of nutrient concentration.
[4] Formulated level (ppm), thiamin inclusion in diet calculated based on an estimated DMI of 3.0 lb·hd^{-1}·d^{-1}.

Table 3. Influence of thiamin and sulfur level on hydrogen sulfide production in lambs fed a 60% DDGS based finishing diet

Treatment[1,2]				
CON	LOW	MED	HIGH	HIGH+S
0.0	0.0	0.0	190.6	75.0
66.7	0.0	112.5	25.0	28.1
71.5	0.0	146.9	71.9	93.8
531.3	375.0	310.5	737.5	475.0
778.1	575.0	759.4	1237.5	1350.0
2200.0[a]	887.5[b]	2200.0[a]	2453.1[a]	2378.1[a]
2390.6[a]	1087.5[b]	1875.0[a]	1906.3[a]	2015.6[a]
2852.6[a]	1418.8[b]	2609.4[a]	2406.3[ab]	2406.3[ab]
3312.5[a]	1531.3[c]	2328.1[abc]	1958.2[bc]	3140.6[ab]
2062.5[a]	3287.5[b]	3275.0[b]	4991.6[c]	3046.9[ab]
4687.5[a]	2662.5[b]	2906.3[b]	6657.8[c]	4390.6[a]
5687.5[a]	2650.0[b]	3843.8[c]	7701.3[d]	4859.4[ac]

[1]Treatments: CON (no supplemental thiamin), LOW (50 mg·hd^{-1}·d^{-1} thiamin), MED (100 mg·hd^{-1}·d^{-1} thiamin), HIGH (150 mg·hd^{-1}·d^{-1} thiamin), and HIGH+S (150 mg·hd^{-1}·d^{-1} thiamin with 0.87% S).
[2] When tube measurement was below 100ppm tube was considered to read 0.
[abc] Means with different superscripts within a row differ $P < 0.10$.

Literature Cited

Loneragan, G, D. Gould, J. Wagner, F. Garry, and M. Thoren. 2005. The magnitude and patterns of ruminal hydrogen sulfide production, blood thiamin concentration, and mean pulmonary arterial pressure in feedlot steers consuming water of different sulfate concentrations. The Bovine Practitioner. 39:16-22.

Niles, G. A., S. Morgan, W. C. Edwards, and D. Lalman. 2002. Effects of dietary sulfur concentrations on the incidence and pathology of polioencephalomalacia in weaned beef calves. Vet. Human Toxicol. 44(2):70-72.

Impacts of integrated pest management of Leafy Spurge (Euphorbia esula) following a 10-year sheep grazing study: A progress report

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The objective of the current study is to determine the most effective combination of grazing and herbicide treatments in combination of bio-control with insects for control of leafy spurge. This report highlights initial findings for an on-going study.

Introduction

Leafy spurge (*Euphorbia esula*) was first reported in North America in 1827 (Kaufman and Kaufman, 2007). Native to central and eastern Europe, leafy spurge was inadvertently introduced in cultivated crop seeds and as an ornamental in the United States. Worldwide introduction has brought leafy spurge to every continent except Australia (Lajeunesse et al., 1997). Leafy spurge is found in 35 states of the U.S. and throughout Canada, thriving in uncultivated areas (Kaufman and Kaufman, 2007). Within the North Great Plains (NGP) region, Liestritz et al. (2004) estimated the direct economic loss from leafy spurge at $37 million with secondary impacts of $83 million.

Biology and Ecology. Leafy spurge is a perennial forb, reaching a height of up to three feet and existing in a variety of habitats (Lajeunesse et al., 1997; Kaufman and Kaufman, 2007). Small, yellow-green flowers develop on like colored bracts (Lajeunesse et al., 1997). Growth begins in early spring with the first period of flower development occurring in late May and June. Additional periods of flowering can occur throughout the growing season.

Root structure plays a key role in successful colonization (Lajeunesse et al., 1997; Kaufman and Kaufman, 2007). Fibrous roots develop thick mats in the upper layer of soil, while taproots descend to 26 feet or more. Specialized root buds can produce a new plant if the top shoot is removed (Dersheid et al., 1985). Lym and Messersmith (1993) found leafy spurge root systems are most cold tolerant in the upper six inches of soil. Cultivation causes root fragmentation that increase root density in the subsequent year. However, but by the third year of cultivation leafy spurge density decreased to 0-30%. Laboratory experiments found leafy spurge root segments of one centimeter could regenerate six percent of the time (Lym and Messersmith, 1987). The diverse and massive root system aids leafy spurge in storing carbohydrates essential for surviving stressful

environmental conditions (Lajeunesse et al., 1997) and early season growth (Dersheid et al., 1985).

Each flowering stem develops pod-like structures filled with seeds (Lajeunesse et al., 1997; Kaufman and Kaufman, 2007), potentially producing up to 140 seeds per stem. Once the pod has dried, it bursts open dispensing seeds up to 15 feet from the parent plant. Seeds can remain viable in the upper layers of the soil for eight years, while deeply buried seeds a longer potential life span. Selleck et al. (1962) found seeds remained viable for up to 13 years. Long distance dispersal relies on transfer of seeds embedded in fur, mud or feces (Lajeunesse et al. 1997).

Herbicide Control. Herbicides provide leafy spurge control at varying levels. Lym and Messersmith (1990) found picloram applied at a rate of two pounds/acre, applied twice, provided 90% control of leafy spurge, while dicamba applied at eight pounds/acre, applied twice, provided 70% control. For long-term control, an annual treatment using picloram and 2,4-D at a rate of 0.25 plus 1 pound/acre reduced leafy spurge density 85-93% after five years (Lym and Messersmith, 1987). Recent studies using picloram found fall was the preferred time of application (Lym and Messersmith, 2006). Annual applications are recommended until 90% control is reached. Lym and Messersmith (2006) found 2,4-D reduced leafy spurge top growth during the season applied. Lym (2000) found 2,4-D did not translocate to leafy spurge roots, thus considered less effective in controlling or killing root growth. Application of 2,4-D is common in areas around water when picloram use is prohibited or when grazing animals may be sensitive to herbicides (Lym and Messersmith (2006).

The use of imazapic, methylated seed oil, and 28% nitrogen at a rate of two ounces plus two pints plus two pints/acre produced 98%, 78%, 94%, and 71% leafy spurge control for nine, 12, 21, and 24 months; respectively, after one treatment (Markle and Lym, 2001). Nitrogen aided in the absorption of imazapic with foliar applications. Markle and Lym (2001) found imazapic alone at a rate of two ounces/acre reduced leafy spurge by 75%, 33%, 74%, and 43% respectively for nine, 12, 21, and 24 months following the one treatment. Treatments were applied for two consecutive years.

Livestock grazing. Grazing with sheep and goats has proven to be successful in controlling leafy spurge. Cattle have an aversion to toxins contained in leafy spurge and can develop scours if enough spurge is consumed (Heemstra et al., 1999). Sheep and goats, however, readily forage on leafy spurge (Walker et al., 1994). Differences in internal organs allow each species to consume different types of forage than cattle (Frost and Launchbaugh, 2003). Sheep are able to consume more forbs due to a large rumen, while a large liver allows goats to more efficiently process toxic compounds.

A reduction in sheep grazing occurs when pastures reach a high-density of leafy spurge. Walker et al. (1994) showed sheep consumed only 51% of the available leafy spurge in a pasture per season. Dahl et al. (2003) found sheep remove only leave and flowering portions of the plant. Grazing by sheep over a four-year period can reduce leafy spurge stem density by 99% (Schauer et al., 2006). Cattle and sheep combined require 5 years of grazing to achieve the same level of control with sheep only when sheep consumed 100% of the carrying capacity. Dahl et al. (2000) found six years of grazing by cattle and sheep is required to reach a 98% level of control. The use of sheep with cattle did not decrease cattle or sheep performance, or change grass and grass-like species production.

In contrast, goats readily graze leafy spurge consuming up to 66% in a single pasture per season (Walker et al., 1994). Goats tend to defoliate leafy spurge rather than consume just flower and leaf parts. Angora goats used at Camp Grafton, ND reduced leafy spurge stem densities by 84.2% and shrubs 91.6% in a four-year period (Sedivec et al., 1995). Sedivec and Maine (1993) found a 57.2% increase in grass and a

44.1% decrease in leafy spurge after two years of grazing with angora goats. Sedivec et al. (1995) found that grass species production increased significantly after three years of grazing.

Biological Controls. Four genera of biological control agents were released in the United States to combat leafy spurge (Hansen et al., 1997). Root boring moths (*Chamaesphecia hungarica*) lay eggs on leafy spurge stems with larvae move downward, burrowing into the roots and killing the plant (Gassman and Tosevski, 1994). Female root-boring beetles (*Oberea erythrocephala*) girdle leafy spurge stems and lay eggs in a cavity. The larvae tunnel downward through the stem to the root area (Schroeder, 1980). Gall midge (*Spurgia esulae*) laid eggs near buds and once the eggs hatch the instars feed on the buds (Pecora et al., 1991). Flea beetle (*Apthona* spp.) adults consume foliage and flowers. The female lay eggs at the base of the stem and once hatched, feed on the shallow, fine roots of leafy spurge (Gassman et al., 1996). Of the four agents released, the flea beetle has had the greatest success with established populations in 18 states (Hansen et al., 1997).

Flea beetles slowly decrease leafy spurge density (Lym and Nelson, 2000). Several subspecies of the leafy spurge flea beetle (*Apthona* spp.) were released in the NGP region. *Apthona nigriscutis* decreased leafy spurge densities by 65% within 53 feet of its release. The reduction in leafy spurge took three to five years. *A. czwalinae* and *A. lacertosa* took four years to reduce leafy spurge densities by 95%. *A. nigriscutis* required a beetle density of 4-8 beetles/yd^2 and *A. czwalinae* and *A. lacertosa* a beetle density of 22.5 beetles/yd^2. *A. czwalinae* was more prolific and dispersed faster from the release site. Hansen et al. (1997) found flea beetles are not suited for release in high-density leafy spurge areas. Lym and Olson (1999) found densities of 60-90 stems/yd^2 were the limit for flea beetle introduction. Soil type also influences flea beetle establishment. Sandy soils reduced flea beetle establishment (Larson et al., 2008), while silt loam, silty clay loam, clay loam, and clay soils with 6-9.5% organic matter had the highest establishment rates (Lym and Olson, 1999). South facing slopes had the highest establishment success.

Combining different control methods can be an effective management tool (Lym, 2005). Integrated pest management systems use site assessment to select the most appropriate control methods based on landowner's budget and site conditions. Multiple control methods can target different parts of the leafy spurge plant and life stages, thus providing better overall control of leafy spurge (Lajeunesse et al., 1997).

Procedures

Study Sites. This study was developed to test different management practices on leafy spurge re-establishment following a long-term sheep grazing study near Mandan, North Dakota at two locations. The first location is owned by the North Dakota State Correctional Center (NDSCC) two miles southwest of Mandan in Morton County on Section 32, T139N, R81W. The second location is operated by the USDA-ARS Northern Great Plains Research Laboratory and three miles south of Mandan in Morton County on the north half of Section 9, T138N, R81W. The NDSCC location contains two replicate blocks and the USDA-ARS one replicate block. Each replicate consists of a 20-acre block subdivided into four 5-acre plots. The treatments were incorporated using a randomized complete block design in each 5-acre plot. Each of the four 5-acre plots represented one of four treatments from a previous study (see Previous Study section for description). Barker and Whitman (1989) classified the vegetation as northern mixed grass prairie comprised of wheatgrass-grama-needlegrass (*Elymus, Bouteloua, Heterostipa*; Shiftlet, 1994).

Previous Study. The current study was designed to study leafy spurge stem density change following different sheep and cattle grazing treatments using a maintenance type program. The study locations were part of a long-term research project studying three different grazing treatments on leafy spurge control, plant

community impacts, and livestock performance. The grazing treatments included cattle only (**CO**), sheep only (**SO**) and cattle and sheep (**CS**); with a non-use treatment as the control (**Ctrl**; Schauer et al., 2006). Grazing occurred from approximately June 1 through October 1 each year or until 50 to 60% disappearance. Leafy spurge stem densities in the **SO** and **CS** grazing treatments were reduced by 99% from the beginning of the trial (1996) to the end (2006) compared to the **Ctrl**. The **SO** treatment required four years and **CS** five years to achieve 99% reduction in leafy spurge. As a note, flea beetles (*Apthona* species) infested all three replicates in 2001, resulting in leafy spurge stem density reduction on the **CO** and **Ctrl** that had not occurred in the first five years, with leafy spurge stem densities reduced by 91% and 89% on the **CO** and **Ctrl**; respectively.

Current Study. The current maintenance study focuses on integrated pest management using grazing, herbicides, and leafy spurge flea beetles. Based on the results from the previous trial, sheep were selected as the control, since they had effectively decreased leafy spurge in a short period and maintained control throughout the duration of the trial. The cattle only treatment was the least effective method of control in the previous trial. Therefore, additional research using a combination of treatments is necessary to determine potential methods of controlling leafy spurge in conjunction with cattle only grazing.

In May 2006, Admire Pro, an insecticide, was applied at 8 ounces/acre to remove spurge beetles from each of the sites. Core samples were taken in July 2006 to confirm the insecticide treatment was successful. All three replicate sites used in the current trial contained two grazing treatments in the four 5-acre pastures and included one sheep only (**SO**) pasture (considered the control and was previously the **SO** pasture) and three cattle only (**CO**) pastures. The three **CO** pastures comprised the previous study's **CO**, **CS**, and **Ctrl** pastures and labeled as such.

Stocking rates were 1.6 AUM/acre for cattle on the **CO**, **CS**, and **Ctrl** treatments, and 1.4 AUM/acre for **SO**. Although stocking rates were design to be the same between treatments, animal equivalent conversions created slightly different rates. Ten ewes were placed on the **SO** treatment on 20 May and removed by 9 October. Two steers were placed on the cattle only **CO, CS**, and **Ctrl** treatments 1 June and removed by 1 October. The target grazing disappearance rate is 50 to 60% of grass and grass-like species. Grazing occurred at all sites in 2007, 2008, and 2009. Sheep depredation by coyotes occurred at the second NDSCC site in June 2009. Sheep were not replaced at that site due to losses.

Each of the **CO**, **CS**, and **CTRL** 5-acre pastures was further divided into 32 - 12 ft by 50 ft sections (192 ft by 100 ft area). The **SO** contained a total eight 12 ft by 50 ft sections.

Eight treatments were studied and included a non-use control (**NU**); insect only **(I)**; 2.4-D only **(2,4D)**; Plateau only **(P)**; 2,4-D and Tordon **(2,4DT)**; 2,4-D and insect **(2,4DI)**; 2,4-D, Tordon, and insect **(2,4DTI)**; and Plateau and insect **(PI)**. The **CO, CS,** and **Ctrl** pastures contained four replicates the eight treatments, while the **SO** one replicate.

Leafy spurge stem density was determined for each treatment prior to livestock grazing each season. Stem counts were obtained by averaging five 2.7 ft^2 quadrats from each treatment replicate.

Tordon (picloram), Plateau (impazapic), and 2,4-D were applied to the treatment plots in 2008. The 2,4-D treatment was applied at 2 quarts/acre in mid-July and the 2,4-D and Tordon treatment applied at rates of 1 quart and 1 pint/acre; respectively, in mid-July. Plateau was applied at the rate of 7 ounces/acre in late September. Herbicide was applied by a hand sprayer. Flea beetles reinvaded all three sites by 2007 and not manually applied with the combination treatments in 2008.

Treatment effect for leafy spurge stem density between treatments was analyzed using SAS (SAS Inst. Inc., Cary, NY) GLM statistical model to compare between treatments and across years. A SAS analysis using a split plot design was used to compare year, block, and grazing treatment affects. When significant differences

occurred ($P \leq 0.05$), Tukey's Honesty Significant Difference was performed to separate differences.

Results

Insects were removed as a treatment from the study due to reinfestation of flea beetles at all three sites. The study was modified to four treatments (three herbicides and one control) with a eight replication pasture in **CO, CS**, and **Ctrl** and two replicated within the **SO** pasture.

Significant changes ($P \leq 0.05$) in leafy spurge stems density occurred between treatments in each of the three cattle grazing treatments (**CO, CS,** and **Ctrl**) in 2009 (Figure 1). The **P** treatment was more effective when compared to the **NU** and **2,4DT** treatments in the **CO** pasture. The **P** treatment reduced ($P \leq 0.05$) leafy spurge density 56.6% and 38.7% greater than the **NU** and **2,4DT**; respectively. Within the **CS** treatments, **P** reduced ($P \leq 0.05$) leafy spurge density by 56.5% and 60.7% compared to the **NU** and **2,4D**; respectively. Leafy spurge density was also best controlled by **P** in the **Ctrl** pasture. The **P** treatment reduced ($P < 0.05$) leafy spurge by 51.5% and 37.2% compared to **2,4D** and **NU**; respectively.

The only other herbicide to show difference in leafy spurge density changes was **2,4D** in the **CO** pasture. Leafy spurge was reduced by 46.2% by **2,4D** compared to the **NU** (Figure 1). Leafy spurge stem density was at 99% control in the **SO** pasture, similar to pre-levels found in the previous study.

Levels of leafy spurge varied between the grazing treatments pastures. Pre-treatment levels of spurge in 2007 were 29.4 times greater in the **Ctrl**, 5.2 times greater in the **CS**, and 29.4 times greater in the **CO** compared to the **SO**. Leafy spurge presence in **SO** was maintained at levels below 1.3%. Comparisons of grazing treatments within 2008 showed **CO** had a higher level ($P = 0.01$) of leafy spurge compared to the **SO** treatments (Figure 1). A difference in leafy spurge stem density levels was found between **Ctrl** and **SO** ($P = 0.007$), **CO** and **CS** ($P = 0.037$), and **CO** and **SO** ($P = 0.047$) in 2009.

Discussion

Plateau at a rate of 7 oz/acre applied in late summer consistently reduced leafy spurge in all three of cattle grazing pastures. Plateau targets the root system and is drawn down into the plant's roots when fall applied (Markle and Lym, 2001). At this time of the growing season in North Dakota, plants draw down available nutrients to aid in over wintering. Markle and Lym (2001) found Imazapic (Plateau) alone reduced leafy spurge by 75% nine months after the first application (mid September), with a decrease in effectiveness to 33% twelve months after the first treatment. The results of this study showed an overall effectiveness rate nine months after the first application of 50.1% compared to **NU** (Figure 2). The level of control was not as high in our study, which may be attributed to the higher number of replications. The Markle and Lym study had four field replicates for each of their herbicide treatments. This study used three blocks with 24 replications contained in each of the blocks among the **CO, CS,** and **Ctrl** pastures, totaling 72 replications for each of the herbicide treatments. The higher number of replications may show a truer level of herbicide effectiveness in field settings.

The 2,4-D treatment applied at 2 qt/ac during the flower growth stage reduced leafy spurge only in the **CO** grazing treatment (Figure 2). Leafy spurge levels increased in the nine months following the first treatment in the **Ctrl** and **CS** treatments. Averaged across all three grazing treatments, a 2.4% decrease in leafy spurge stem density occurred. Lym and Messersmith (2006) found a 20% reduction in leafy spurge using 2,4-D applied at a rate of 1 qt/ac and a reapplication of 1pt/acre twelve months following the initial application. Their timing for herbicide application was June when flowering of leafy spurge was at maximum. Our application timing was in mid-July during a later period of flowering. The lower control levels of 2,4-D compared to Plateau may reflect a lower 2,4-D control due to application time (Lym, 2000).

The 2,4-D plus Tordon treatment reduced leafy spurge from

8.2% to 29.2% with an average reduction between all grazing treatments 17.9% (Figure 2). Lym and Messersmith (2006) found leafy spurge reduction rates of 50% 12 months following the first application. Their application rate was also 1 qt/ac plus 1 pt/acre of 2,4-D and Tordon; respectively. Their application of the 2,4-D plus Tordon mix occurred in June, which they determined was the optimal treatment timing for this herbicide combination. Our application of the 2,4-D plus Tordon was mid-July. Earlier spraying of herbicides such as 2,4-D and Tordon appear to weaken leafy spurge at a time when a large portion of its nutrients and energy are used for seed production and plant growth (Lym and Messersmith, 1985). Later in the season, when leafy spurge has attained maturity, it is less vulnerable to these herbicide applications.

Flea beetles may have aided in controlling leafy spurge to a certain extent. Flea beetle presence was noted in 2007, 2008, and 2009; however, counts were not collected to determine beetle density. As noted by Lym and Nelson (2000) effective flea beetle densities range from 4-22.5 beetles/yd^2, and once introduced, individuals may take 3-5 years to control leafy spurge. Once established, the combination of flea beetles and herbicides may compliment each by weakening both the reproductive cycle and root systems of leafy spurge.

The previous study's grazing treatments appear to have an ongoing impact on leafy spurge levels. The 2007 levels of leafy spurge were lower in the **CS** grazing treatment compared to the **CO** and **Ctrl.** Stem count comparisons in 2008 showed a continued trend with higher levels of leafy spurge in **CO** treatments compared to **SO**. Leafy spurge counts in 2009 (Figure 1) continue the trend of higher levels of spurge in the **CO** treatments compared to the **SO** and lower levels of leafy spurge within the **CS** pastures compared to the **CO** and **Ctrl** treatments. In the previous study the **CO** treatment was least responsive to leafy spurge control by grazing (Schauer et al. 2006), while the **CS** treatment was second in effectively controlling leafy spurge. The **SO** pastures have consistently shown very low spurge numbers in both studies, confirming sheep are an effective method to control leafy spurge and maintain infestations.

Additional research is required to determine if herbicides will control leafy spurge within the cattle grazing treatments at levels comparable to sheep grazing.

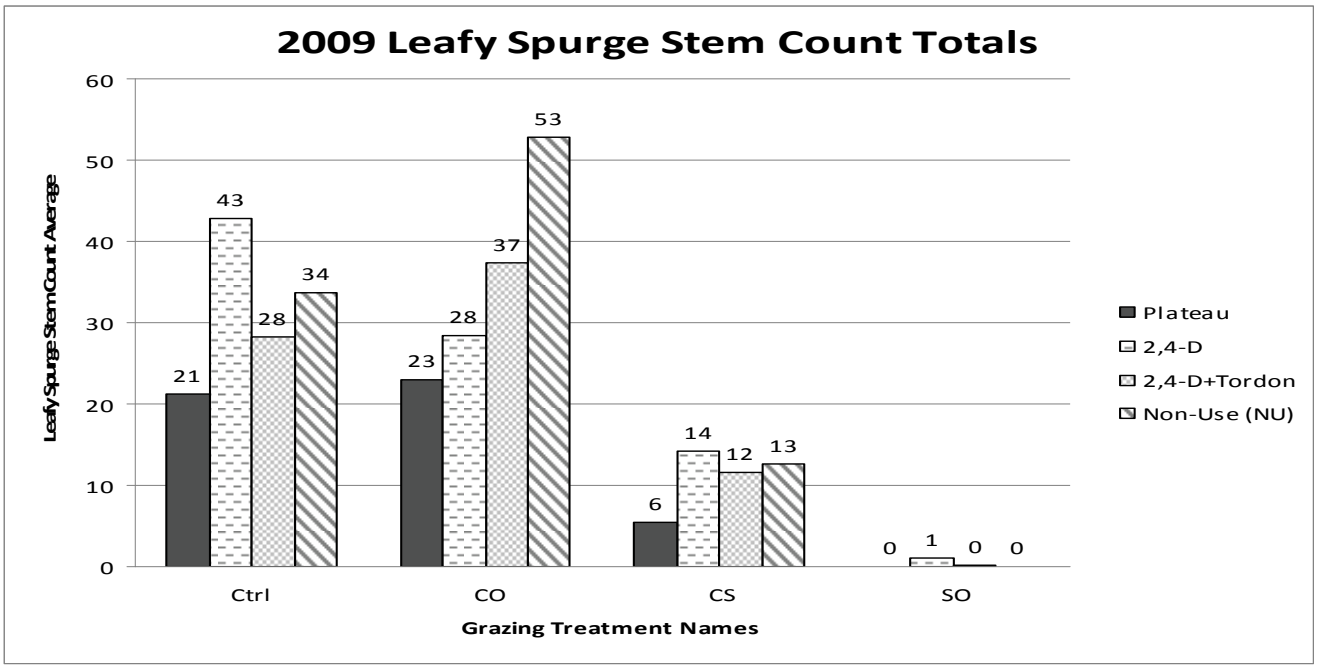

Figure 1. Average leafy spurge stem count within the treatments for 2009. Averages based on the total of five 2.7 ft^2 quadrats per herbicide treatment. **CO**, **CS**, and **Ctrl** are the three cattle only treatments for the current study with two steers per 5 acre pasture. The **SO** treatment has ten ewes per pasture and is the control for the study. **SO** (sheep only) shows the best overall control of leafy spurge.

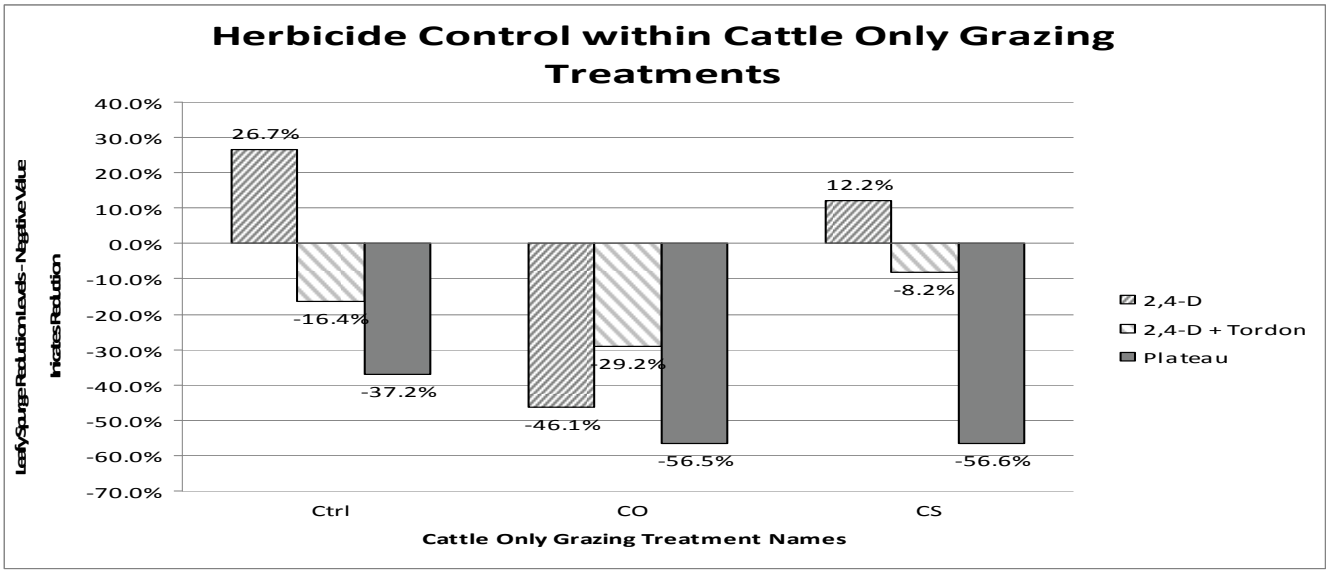

Figure 2. Effectiveness of herbicides nine months follwing the first application. Negative values indicate a reduction in leafy spurge. The three cattle only treatments are shown above as **Ctnl**, **CO**, and **CS**. Overall herbicide control was most effective within the **CO** treatments.

Literature Cited

Barker, W.T. and W.C. Whitman. 1989. Vegetation of the northern Great Plains. Rangelands 10:266-272.

Dahl, J.D., K.K. Sedivec, T.C. Faller, J.F. Karn, P.E. Nyren, and J. Olson. 2003. Multi-species grazing and single species grazing on leafy spurge infested rangeland (seven-year summary). Sheep Day Report. North Dakota State University Hettinger Extension Station.

Derscheid, L.A. L.J. Wrage, and W.E. Arnold. 1985. Cultural control of leafy spurge. In *Leafy spurge, Monograph Series of the Weed Science Society of America.* Editor. Alan K. Watson. Chapter 6 (3) 57-64.

Frost, R.A. and K.L. Launchbaugh. 2003. Prescription grazing for rangeland weed management: A new look at an old tool. Rangelands. 25(6) 43-47.

Gassman, A., D. Schroeder, E. Maw, and G. Sommer. 1996. Biology, ecology, and host specificity of European *Anthona* spp. (Coleoptera, Chrysomelidae) used as biocontrol agents for leafy spurge, *Euphorbia esula* (Euphorbiaceae) in North America. Biological Controls. 6: 105-113.

Gassman, A. and I. Tosevski. 1994. Biology and the host specificity of *Chamaesphecia hungarica* and *Ch. Astatiformis* (Lep.: Sesiidae), two candidates for the biological control of leafy spurge, *Euphorbia esula* (Euphorbiaceae), in North America. Entomophaga. 39:237-245.

Hansen, R.W., R.D. Richard, P.E. Parker, and L.E. Wendel. 1997. Distribution of biological control agents of leafy spurge (*Euphorbia esula*) in the United States: 1988-1996. Biological control. 10:129-142.

Heemstra, J.M., S.L. Kronberg, R.D. Neiger, and R.J. Pruitt. 1999. Behavioral, nutritional, and toxicological responses of cattle to ensiled leafy spurge. J. Anim. Sci. 77:600-610.

Kaufman S.R. and W. Kaufman. 2007. A Field Guide to Individual Species. In *Invasive Plants:Guide to Identification and the Impacts and Control of Common North American Species.* Stackpole Books, Mechanicsburg, PA. 458 p.

Lajeunesse, S. R. Sheley, R. Lym, D. Cooksey, C. Duncan, J. Lacey, N. Rees, and M. Ferrell. 1997. Leafy spurge: Biology, ecology, and management. Montana State University Extension Service EB-134. 25 p.

Larson, D.L., J.B. Grace, and J.L. Larson. 2008. Long-term dynamics of leafy spurge (*Euphorbia esula*) and its biocontrol agent, flea beetles in the genus Aphthona. Biological Control. 47:250-256.

Liestritz, F.L. D.A. Bangsund, and N.M. Hodur. 2004. Assessing the economic impact of invasive weeds: the case of leafy spurge (*Euphorbia esula*). Weed Technology. 18:1392-1395.

Lym, R.G. 2000. Leafy spurge (*Euphorbia esula*) control with glyphosate plus 2,4-D. Journal of Range Management. 53(1):68-72.

Lym, R.G. 2005. Integration of biological control agents with other weed management technologies: Successes from the leafy spurge (*Euphorbia esula*) IPM program. Biological Control. 35:366-375.

Lym, R.G. and C.B. Carlson. 2002. Effect of leafy spurge (*Euphorbia esula*) genotype on feeding damage and repro duction of Aphthona spp.: Implications for biological weed control. Biological Control. 23:127-133.

Lym, R.G. and C. G. Messersmith. 1985. Leafy Spurge Control with Herbicides in North Dakota: 20-Year Summary. Journal of Range Management. 38(2):149-153.

Lym, R.G. and C. G. Messersmith. 1987. Leafy spurge control and herbicide residue from annual picloram and 2,4 -D application. Journal of Range Management. 40 (3):194-198.

Lym, R.G. and C.G. Messersmith. 1990. Effect of temperature on picloram absorption and translocation in leafy spurge (*Euphorbia esula*). Weed Science. 38:471-474.

Lym, R.G. and C.G. Messersmith. 1993. Fall cultivation and fertilization to reduce winter hardiness of leafy spurge (*Euphorbia esula*). Weed Science. 41:441 -446.

Lym, R.G. and C.G. Messersmith. 2006. Leafy spurge identification and chemical control. North Dakota State University Extension publication W-765.

Lym, R.G. and J.A. Nelson. 2000. Biological Control of leafy spurge (*Euphorbia esula*) with Aphthona spp. along railroad right-of-ways. Weed Technology. 14:642-646.

Lym, R.G. and D.L. Olson. 1999. Leafy Spurge control using flea beetles (Aphthona spp.). North Dakota State University Extension Bulletin W-1183.

Markle, D.M. and R.G. Lym. 2001. Leafy spurge (*Euphorbia esula*) control and herbage production with imazapic. Weed Technology. 15:474-480.

Pecora, . R.W. Pemberton, M. Stazi, and G.R. Johnson. 1991. Host specificity of *Spurgia esulae* Gagne (Diptera: Cecidomyiidae), a gall midge introduced into the United States for control of leafy spurge (*Euphorbia esula* L. "complex"). Environmental Entomology. 20:282-287.

Schauer, C.S., K.K. Sedivec, T.C. Faller, S. Kronberg, and D.M. Stecher. 2006. Multispecies grazing and single-species grazing on leafy spurge infected rangeland (Ten-year summary). 2006 Sheep and Beef Report. North Dakota State University Hettinger Extension Station.

Schroeder, D. 1980. Investigations on *Oberea erythrocephala* (Schrank) (Col.:Cerambycidae), a possible biocontrol agent of leafy spurge, *Euphorbia* spp. (Euphorbiacea), in Canada. Entomology. 90:237-254.

Sedivec, K.K. W.T. Barker, and C.W. Prosser. 1995. Intensive grazing of angora goats on leafy spurge infested rangeland. Leafy Spurge Symposium. Fargo, ND. pp. 34-36.

Sedivec, K.K. and R.P. Maine. 1993. Angora goat grazing as a biological control for leafy spurge: A three year summary. Proceedings of Great Plains Agricultural Council Leafy Spurge Task Force Symposium. pp. 1-5.

Selleck, G.W., R.T. Coupland, and L. Frankton. 1962. Leafy spurge in Saskatchewan. Ecology Monographs. 32:1-29.

Shiflet, T,N, 1994. Rangeland cover types of the United States. Society for Range Management. Denver, CO. 152 pp.

Walker, J.W. S.L. Kronberg, S.L. Al-Rowaily, and N.E. West. 1994. Comparison of sheep and goat preferences for leafy spurge. Journal of Range Management. 47(6):429-434.

Growth and development of blood vessels in maternal placenta during early pregnancy in sheep[1]

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Data from our study may help to identify factors that can be used therapeutically to restore normal placental vascular function and blood flow to rescue compromised pregnancies. In addition, these data will help to compare the patterns of vascularization and expression of angiogenic factors, and timing of angiogenesis initiation in compromised pregnancies vs. normal pregnancy in future studies.

Summary

Placental vascular development (angiogenesis) is critical for placental function and thus for normal embryonic/fetal growth and development. Specific environmental factors or use of assisted reproductive techniques may result in poor placental blood vessel growth and development (angiogenesis), which may contribute to embryonic losses and/or fetal growth retardation. To provide a description of normal placental angiogenesis, uterine tissues were collected on days 14, 16, 18, 20, 22, 24, 26, 28, and 30 after mating and on day 10 after estrus (nonpregnant controls). To determine vascular development in the endometrium, we used histochemistry and/or immunohistochemistry followed by image analysis. Compared to controls, several measurements of vascularity increased ($P<0.001$) including vascular labeling index (LI; proportion of proliferating cells), the tissue area occupied by capillaries, area per capillary (capillary size), total capillary circumference per unit of tissue area and expression of factor VIII (marker of blood vessels), but capillary number decreased ($P<0.001$) in endometrium. These data indicate that endometrial angiogenesis, manifested by increased vascularity is initiated very early in pregnancy. This more complete description of early placental angiogenesis will provide the foundation for determining whether placental vascular development is altered in compromised pregnancies.

Introduction

During pregnancy, vascular development or angiogenesis parallels the growth of uterine and placental tissues to support fetal growth and development (Reynolds and Redmer, 2001; Reynolds et al. 2005a,b,c, 2006). Inadequate vascular growth during early pregnancy may be associated with inadequate uterine and umbilical blood flow, which directly affects transport of nutrients to the embryo/fetus. The consequences of inadequate placental vascular development include compromised implantation, spontaneous abortion/embryonic loss, defective formation of the placenta, and

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altered fetal growth and development resulting in intrauterine growth restriction (IUGR) potentially leading to reduced lifelong health and productivity of the offspring (Wallace et al. 2002; Reynolds et al. 2005a,b,c, 2010; Sherer and Abulafia, 2001; Torry et al. 2004; Demir et al. 2007).

Most of embryonic loss occurs in early pregnancy reaching ≥30% of embryos lost in most mammalian species and possibly ≥50% in humans (Reynolds and Redmer, 2001). These are astonishing figures and highlight the early part as a critical period of pregnancy. High embryonic loss may appear as a consequence of a variety of negative effects, and thus establishes the need to analyze angiogenesis in both normal pregnancies and compromised pregnancies, such as those generated from assisted reproductive technologies (ART), or affected by environmental factors such as malnutrition or other stress.

Angiogenesis is the formation of new blood vessels from preexisting vasculature, and it is a critical process for the growth and development of all tissues, including the placenta (Reynolds and Redmer, 1992, 1998; Borowicz et al. 2007; Reynolds et al. 2010). Growth of the tissue is associated with the high metabolic demands (Reynolds and Redmer, 1998). The placenta, which serves as the organ of exchange between maternal and fetal systems, represents a very fast growing tissue with a high metabolic demand that requires a dynamic angiogenic process from early to late pregnancy to support its growth and function (Mayhew 2002; Torry et al. 2004; Reynolds and Redmer, 1995, 2001; Redmer et al. 2004; Reynolds et al. 2005a,b,c, 2010). Angiogenesis is regulated by numerous angiogenic factors including vascular endothelial growth factor (VEGF) family, fibroblast growth factor 2 (FGF2, also known as basic fibroblast growth factor), angiopoietins (ANGPT), nitric oxide (NO) system and other factors (Reynolds and Redmer, 1998; Reynolds et al. 2005a,b,c, 2010). Moreover, placental angiogenesis is abnormal at mid to late period in compromised pregnancies (Reynolds et al. 2005a,b,c, 2006, 2010; Meyhew et al. 2004; Burton et al. 2009). Very limited information is available concerning changes in vascular development and expression of angiogenic factors during early pregnancy.

We hypothesized that proliferation of vascular cells, and vascular growth and development will begin to change very early in pregnancy. Therefore, the purpose of the present study was to determine the pattern of vascular cell proliferation and vascular growth. These data will serve as an important reference to study placental developmental defects in compromised pregnancies in the future.

Procedures

Animals and tissue collection. The Institutional Animal Care and Use Committee at NDSU approved all animal procedures in this study. Gravid uteri were obtained from crossbred Western Range (primarily Rambouillet, Targhee, and Columbia crossbreeds) ewes (n = 6 to 8 per day) on days 14, 16, 18, 20, 22, 24, 26, 28, and 30 after mating (day of mating = day 0), and also from mid-luteal, nonpregnant (day 10 after estrus; n = 8) control ewes. For immunohistochemical staining, at tissue collection, specimen pins were inserted completely through the uterus and fetal membranes at the level of the external intercornual bifurcation to maintain specimen morphology; cross sections of the entire gravid uterus (0.5 cm thick) were obtained using a Stadie-Riggs microtome blade, immersion-fixed in Carnoy's solution, and embedded in paraffin.

Histochemistry and immunohistochemistry. Histochemical and immunohistochemical procedures were used as described before (Borowicz et al. 2007; Grazul-Bilska et al. 2008, 2009). Briefly, paraffin-embedded tissues were sectioned at 5 μm, mounted onto glass slides, rinsed several times in PBS containing Triton-X100 (0.3%, v/v), and treated for 20 min with blocking buffer [PBS containing normal goat serum (2%, vol/vol)]. The tissue sections were then incubated with specific primary antibody for proliferating cell nuclear antigen (PCNA; 1:500 dilution; monoclonal mouse; Zymed, San Francisco, CA) or factor VIII (1:100; rabbit polyclonal antibody, Sigma, St. Louis, MO) overnight at 4˚ C. Primary PCNA or factor VIII antibodies were detected by using biotin-

labeled secondary anti-mouse or anti-rabbit antibodies, respectively, and the ABC method (Vector Laboratories Burlingame, CA). For PCNA staining, the sections were then counterstained with hematoxylin and periodic acid-Schiff's reagent (H plus PAS). Control sections were incubated with normal mouse or rabbit serum in place of PCNA or factor VIIII primary antibody, respectively.

Image analysis. Image analysis was performed as described in detail before (Grazul-Bilska et al. 2009; Borowicz et al. 2007). Images of randomly chosen areas of intercaruncular (ICAR) and caruncular (CAR; 5-10 per uterine section/sheep; 0.025 mm^2 per field) stained for PCNA or factor VIII were taken at 600x (PCNA) or 400x (factor VIII) magnification, using an Eclipse E600 Nikon microscope and digital camera. Vascular labeling index (LI; proportion of proliferating cells within blood vessels), vascularity, and relative expression of factor VIII were determined by using computerized image analysis (Image-Pro Plus, version 5.0; Media Cybernatics, Houston, TX). The following vascularity measurements were determined for endometrial CAR and ICAR: the number of proliferating nuclei along with the total number of nuclei within blood vessels per tissue area to determine vascular LI, capillary area density (CAD; total area occupied by capillaries expressed as a proportion per unit of tissue area), capillary number density (CND; total number of capillaries per unit of tissue area), capillary surface density (CSD; total capillary circumference per unit of tissue area), area per capillary (APC; average cross-sectional area per capillary, which represents average capillary size) and the percentage of the total tissue area that exhibited positive staining for factor VIII.

Statistical analysis. Data were analyzed using the general linear models (GLM) procedure of SAS, with the main effect of day of pregnancy (SAS, 2008), and are presented as means ± SEM. When the F-test was significant ($P<0.05$), differences between specific means were evaluated by using the least significant differences test (Kirk 1982).

Results

Proliferating cells marked by PCNA, and the network of blood vessels marked by H plus PAS staining or factor VIII were localized in CAR and ICAR regions of endometrial tissues in nonpregnant and pregnant animals. By day 18 after mating, the endometrial luminal epithelium was beginning to flatten, becoming low cuboidal to squamous, compared with that observed on earlier days of pregnancy (days 14 and 16), which were primarily columnar. In addition, by day 18, the subepithelial capillary plexus, which we have previously described (Reynolds and Redmer, 1992; Reynolds et al. 2005a), was beginning to form, and by day 28 it was quite well developed. Extensive vascularization of the endometrial CAR and ICAR tissues began as early as day 18 and was extensive by day 28 after mating. In addition, in some of the ewes, microscopic fetal and maternal placental villi were evident by day 30 and were beginning to vascularize. In contrast, on days 14 and 16 after mating, these pregnancy-induced changes in endometrial morphology or vascular development were not yet evident. The pattern of changes in vascularity and vascular development was similar in CAR and ICAR, therefore, data for vascular measurements including LI, CAD, CND, CSD and APC were combined for CAR and ICAR. Several measurements of vascular growth in CAR and ICAR areas including LI in the blood vessel (Fig. 1A), CAD (Fig. 1B), CND (Fig. 1C), CSD (Fig. 1D), APC (Fig. 1E), and expression of factor VIIII (Fig. 1F) changed dramatically ($P<0.0001$-0.05) compared to nonpregnant controls or from day 14 to day 30 of pregnancy. Compared to nonpregnant controls, vascular LI increased ($P<0.0001$) 7 to 20-fold on days 14 to 20, and 23 to 34-fold on days 22 to 30 of pregnancy (Fig. 1A). Vascular LI for the non-pregnant controls was 0.71 ± 0.12% in CAR and ICAR. Compared to nonpregnant controls, CAD increased ($P<0.001$) 1.3-fold on day 16 and 1.5 to 1.9-fold on days 20 to 30 (Fig. 1B). Compared to nonpregnant controls, CND remained unchanged on days 14 to 20, and then decreased ($P<0.0001$) on days 22 to 30 of pregnancy (Fig. 1C). Compared to nonpregnant controls, CSD

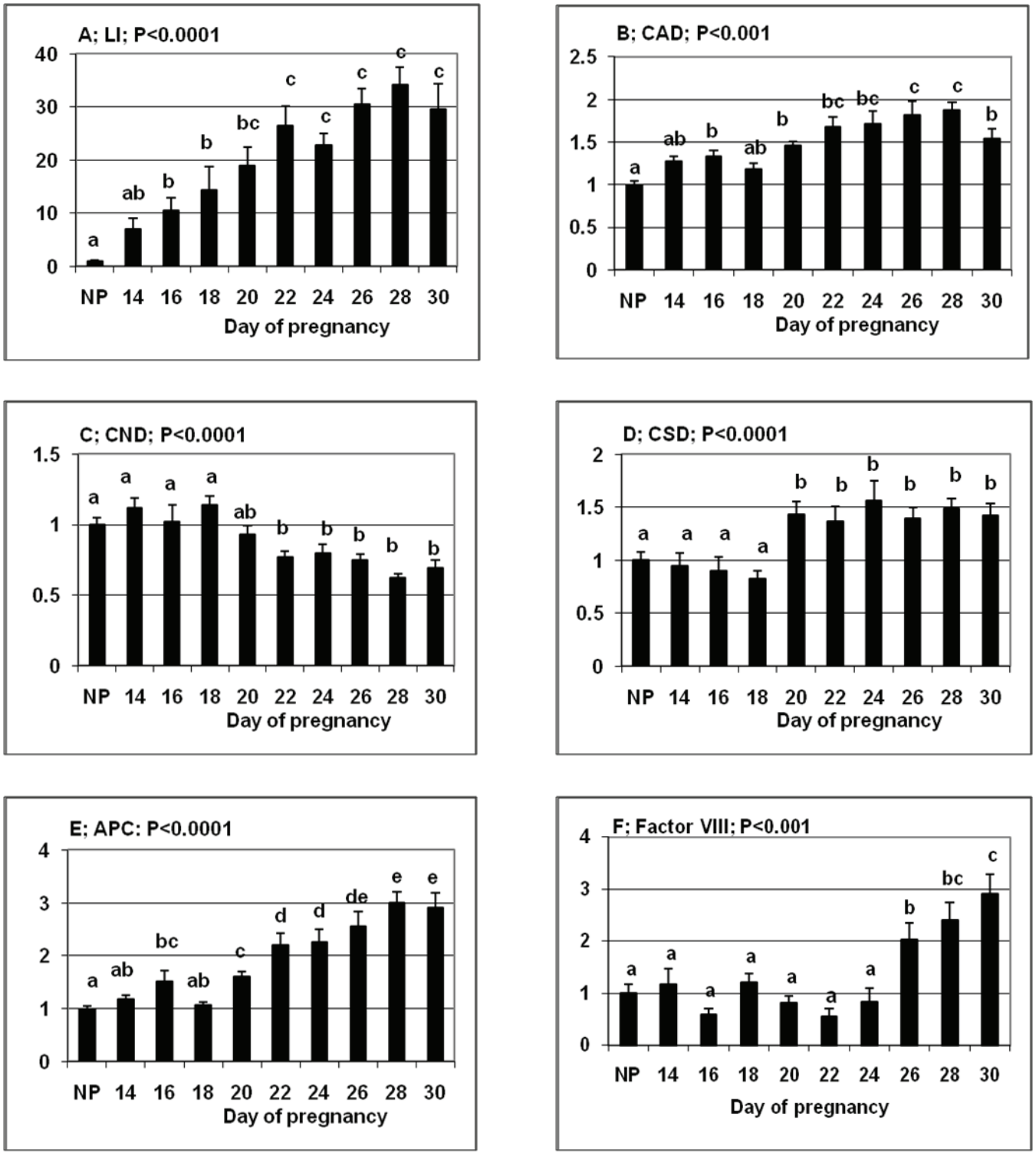

Fig. 1. Measurements of vascularity including vascular labeling index (LI; A), capillary area density (CDA; B), capillary number density (CND; C), capillary surface density (CSD; D), area per capillary (APC; E), and factor VIIII (F) for nonpregnant (NP) controls and on days 14 to 30 of pregnancy. [a,b,c,d,e]P<0.0001-0.001; values ± SEM with different superscripts differ within specific measurement. Data are expressed as a fold-change compared to NP control arbitrary set as 1.

increased (P<0.0001) 1.4 to 1.6-fold on days 20 to 30 of pregnancy (Fig. 1D). Compared to nonpregnant controls, APC increased (P<0.0001) 1.5-fold on days 16 and 22, and 2.2 to 3-fold on days 22 to 30 of pregnancy (Fig. 1E). Area per capillary in non-pregnant controls was 50 ± 3 μm^2. Compared to nonpregnant controls, expression of factor VIII increased (P<0.001) 2 to 3-fold on days 26 to 30 of pregnancy (Fig. 1F).

Labeling index was positively correlated with CAD (r^2 = 0.454; P<0.002), APC (r^2 = 0.577; P< 0.0001) and expression of factor VIII (r^2 = 0.486; P<0.001), and negatively correlated with CND (r^2 = -0.472; P<0.001).

Discussion

Uterine and placental growth, including vascular development is critical for successful pregnancy and production of healthy offspring. For the placenta of many mammalian species, a close relationship exists between fetal weight, placental size and vascularity, and uterine and umbilical blood flows (Reynolds et al. 2005a,b,c). Therefore, the study of normal uterine and placental development, as presented here, is critical to establish the patterns of vascular growth and expression of angiogenic factors that will help to determine the causes and consequences of pregnancy failures in compromised pregnancies, and also to establish therapeutic strategies to rescue compromised pregnancies (Reynolds et al. 2006, 2010; Burton et al. 2009).

The present study demonstrated that angiogenesis is initiated very early in pregnancy, since an increase in vascular LI was first observed on day 14 of pregnancy, and several measurements of angiogenesis including CAD, CSD and APC, and expression of several angiogenic factors increased by day 16 of pregnancy. The most dramatic increase was observed for vascular LI during days 14 to 20 of pregnancy and this was maintained until day 30. This enhanced vascular cell proliferation likely allows for rapid blood vessel growth, which was reflected by the increase of CAD, CSD and APC seen in endometrium. Thus, the time of the major increase in vascular cell proliferation corresponds to the period of maternal recognition of pregnancy, initial attachment/implantation of fetal membranes to uterine epithelium and initiation of placental growth and development (Bowen and Burghardt, 2000; Spencer et al. 2004, 2007, 2008; Bazer et al. 2009). Numerous factors of fetal and uterine origin have been identified in the maternal recognition of pregnancy and implantation in sheep including interferon (IFN)-τ, glycoproteins, steroid hormones, prostaglandins, osteopontin, and growth factors and their receptors (Bowen and Burghardt, 2000; Spencer et al. 2004; Cammas et al. 2006; Weems et al. 2006; Bazer et al., 2009a,b). Some of these factors (e.g., FGF, HIF, prostaglandins, osteopontin) have been recognized as angiogenic factors (Reynolds and Redmer, 2001; Reynolds et al. 2002, 2005a,b, 2006; Bazer et al. 2009a,b). Thus, initiation of angiogenesis seems to be directly associated with maternal recognition of pregnancy and implantation. However, further study should be undertaken to identify which factors are involved in both pregnancy recognition signaling and initiation of angiogenesis.

Although the importance of vasculogenesis and angiogenesis during early pregnancy is well recognized (Pfarrer et al. 2001, 2006; Sherer and Abulafia, 2001; Mayhew 2002; Demir et al. 2007; Reynolds et al. 2010), limited data are available concerning the timing of initiation of placental angiogenesis. Our previous studies have demonstrated that endometrial cellular proliferation and microvascular volume increased from day 12 to day 18 or 24 of pregnancy and remained elevated, and the general pattern of changes in vascular architecture in maternal placenta during early pregnancy in sheep have been reported (Reynolds and Redmer, 1992, Zheng et al. 1996). In the current, more detailed study, we observed increased maternal placental vascular cell proliferation as early as day 14, increased expression of angiogenic factors by days 14 to 16, and changes in vascular architecture as early as day 18 of pregnancy. These minor discrepancies between previous and current experiments are likely due to the more sensitive and precise techniques we used for determination of vascular changes in this study (Borowicz et al. 2007).

For humans, angiogenesis associated with extensive vascular remodeling in endometrium is initiated during the first weeks of pregnancy and is completed around 20 weeks of gestation (Huppertz and Peeters, 2005; Arroyo and Winn, 2008). Increased endometrial endothelial cell proliferation and blood vessel diameter were observed on week 2 of pregnancy in the marmoset (Rowe et al. 2004). In rats, a dramatic increase in endothelial cell proliferation index was observed as early as on day 3 of pregnancy; however, endothelial cell density did not change in endometrium (Goodger and Rogers, 1995). Based on the results presented above, it is reasonable to postulate that maternal recognition of pregnancy and implantation involves initiation of angiogenesis manifested first by increased vascular cell proliferation followed by enhanced vascularization and expression of angiogenic factors in maternal placenta.

The pattern of blood vessel growth during early pregnancy in this study resembles the pattern from mid to late pregnancy described before (Borowicz et al. 2007) except that during early pregnancy the number of blood vessels per unit tissue area decreased perhaps reflecting the large increase in capillary size. As pregnancy progresses, both area per capillary and number of blood vessels per tissue area increased moderately (Borowicz et al. 2007). Additionally, during early pregnancy, the expression pattern of changes for some of angiogenic factors differs (e.g., NOS3, ANGPT1, ANGPT2, HIF) and for some resembles (e.g., VEGF, VEGFR1, VEGFR2, ANGPTR Tie-2, FGF2) the pattern from mid to late pregnancy (Borowicz et al. 2007). This indicates a different role of specific angiogenic factor in regulation of blood vessel growth and function at different stages of pregnancy in sheep. However, this subject should be further investigated.

Implications

In summary, in this study, we have shown changes in vascular architecture, vascular cell proliferation and expression of factor VIIII. Since the pattern of changes in vascular development parallels expression of several angiogenic factors (Grazul-Bilska et al., 2009b), it indicates a complex regulation of these angiogenic processes. In addition, we know the increase of blood flow is closely associated with angiogenesis (Reynolds et al. 1984; Reynolds and Redmer, 1995). Thus, angiogenesis is crucial in developing and building the placental life line between the maternal and fetal systems and when affected, fetal growth and development are also affected (Carter and Charnock-Jones, 2001; Wulff et al. 2003; Arroyo et al. 2008; Fraser and Duncan, 2009). In fact, abnormalities of placental angiogenesis and expression of angiogenic factors are associated with a variety of compromised pregnancies, including those resulting from environmental stress, maternal iron deficiency, undernutrition or overnutrition, pre-eclampsia, gestational diabetes, application of assisted technologies, and other factors (Reynolds et al. 2006, 2010; Arnold et al. 2008; Stillerman et al. 2008). Therefore, data from our study may help to identify factors that can be used therapeutically to restore normal placental vascular function and blood flow to rescue compromised pregnancies. In addition, these data will help to compare the patterns of vascularization and expression of angiogenic factors, and timing of angiogenesis initiation in compromised pregnancies vs. normal pregnancy in future studies.

Literature Cited

Arnold, D. R., A. L. Fortier, R. Lefebvre, M. A. Miglino, C. Pfarrer, and L. C. Smith. 2008. Placental insufficiencies in cloned animals - a workshop report. Placenta 29, Suppl A:S108-110.

Arroyo, J. A., and V. D. Winn. 2008. Vasculogenesis and angiogenesis in the IUGR placenta. Semin Perinatol. 32:172-177.

Bazer. F. W., T. E. Spencer, and G. A. Johnson. 2009a. Interferons and uterine receptivity. Semin Reprod Med. 27:90-102.

Bazer. F. W., T. E. Spencer, G. A. Johnson, R. C. Burghardt, and G. Wu. 2009b. Comparative aspects of implantation. Reproduction 138:195-209.

Borowicz , P. P., D. R. Arnold, M. L. Johnson, A. T. Grazul -Bilska, D. A. Redmer, and L. P. Reynolds. 2007. Placental growth throughout the last two-thirds of pregnancy in sheep: vascular development and angiogenic factor expression. Biol. Reprod. 76:259-67.

Bowen, J. A., and R. C. Burghardt. 2000. Cellular mechanisms of implantation in domestic farm animals. Semin Cell Dev Biol. 11:93-104.

Burton, G. J., D. S. Charnock-Jones, and E. Jauniaux. 2009. Regulation of vascular growth and function in the human placenta. Reproduction 138:895-902.

Cammas, L., P. Reinaud, N. Bordas, O. Dubois, G. Germain, and G. Charpigny. 2006. Developmental regulation of prostacyclin synthase and prostacyclin receptors in the ovine uterus and conceptus during the peri-implantation period. Reproduction 131:917-927.

Carter, A. M., and D. S. Charnock Jones DS. 2001. Angiogenesis and blood flow: implications for pathobiology--a workshop report. Placenta 22 Suppl A:S66-68.

Demir, R., Y. Seval, and B. Huppertz. 2007. Vasculogenesis and angiogenesis in the early human placenta. Acta Histochem. 109:257-265.

Dunk, C., M. Shams, S. Nijjar, M. Rhaman, Y. Qiu, B. Bussolati, and A. Ahmed. 2000. Angiopoietin-1 and angiopoietin-2 activate trophoblast Tie-2 to promote growth and migration during placental development. Am J Pathol. 156:2185-2199.

Fraser, H.M., and W. C. Duncan. 2008. SRB Reproduction, Fertility and Development Award Lecture_Regulation and manipulation of angiogenesis in the ovary and endometrium. Reprod Fertil Dev. 21:377-392.

Goodger, A.M., and P. A. Rogers. 1995. Blood vessel growth and endothelial cell density in rat endometrium. J Reprod Fertil. 105:259-261.

Grazul-Bilska, A. T., J. Banerjee, I. Yazici, E. Borowczyk, J. J. Bilski, R. K. Sharma, M. Siemionov, and T. Falcone. Morphology and function of cryopreserved whole ovine ovaries after heterotopic autotransplantation. Reproductive Biology and Endocrinology, 6: 16; 2008.

Grazul-Bilska, A.T., J. S. Caton, W. Arndt, K. Burchill, C. Thorson, E. Borowczyk, J. J. Bilski, D. A. Redmer, L. P. Reynolds, and K. A. Vonnahme. 2009a. Cellular proliferation in ovine fetal ovaries: Effects of energy restrictions and selenium in maternal diet. Reproduction 137:699-707.

Grazul-Bilska, A.T., P. P. Borowicz, M. L Johnson, R. Wroblewski, M. A Minten, D. A Redmer, and L. P. Reynolds. 2009b. Relationship Between Vascular Growth and Expression of Angiogenic Factors in Uterine Tissues During Early Pregnancy in Sheep. Abstract, 42th Annual Society for the Study of Reproduction meeting.

Huppertz, B., and Peeters, L.L. 2005. Vascular biology in implantation and placentation. Angiogenesis 8:157-167.

Kirk, R.E. 1982. Experimental Design: Procedures for the Behavioral Sciences, 2nd Edn., Brooks/Cole, Belmont, CA.

Mayhew TM. 2002. Fetoplacental angiogenesis during gestation is biphasic, longitudinal and occurs by proliferation and remodelling of vascular endothelial cells. Placenta 23:742-750.

Mayhew, T.M., D.S. Charnock-Jones, and P. Kaufmann. 2004. Aspects of human fetoplacental vasculogenesis and angiogenesis. III. Changes in complicated pregnancies. Placenta 25:127-139.

Pfarrer, C., B. Ebert, M.A. Miglino, K. Klisch, and R. Leiser. 2001. The three-dimensional feto-maternal vascular interrelationship during early bovine placental development: a scanning electron microscopical study. J. Anat. 198:591-602.

Pfarrer, C.D., S.D. Ruziwa, H. Winther, H. Callesen, R. Leiser, D. Schams, and V. Dantzer. 2006.

Localization of vascular endothelial growth factor (VEGF) and its receptors VEGFR-1 and VEGFR-2 in bovine placentomes from implantation until term. Placenta 27:889-898.

Redmer, D. A., J. M. Wallace, and L. P. Reynolds. 2004. Effect of nutrient intake during pregnancy on fetal and placental growth and vascular development. Dom. Anim. Endocrinol. 27:199-217.

Reynolds, L.P., J.S. Caton, D. A. Redmer, A. T. Grazul-Bilska, K. A. Vonnahme, P. P. Borowicz, J. S. Luther, J. M. Wallace, G. Wu, and T.E. Spencer. 2006. Evidence for altered placental blood flow and vascularity in compromised pregnancies. J. Physiol. 572:51-58.

Reynolds, L. P., M. E. Biondini, P. P. Borowicz, A. T. Grazul-Bilska, K. A. Vonnahme, J. S. Caton, and D. A. Redmer. 2005a. Functional significance of developmental changes in placental microvascular architecture: The sheep as a model. Endothelium 12:11-19.

Reynolds, L. P., P. P. Borowicz, J. S. Caton, K. A. Vonnahme, J. S. Luther, D. S. Buchanan, S. A. Hafez, A. T. Grazul-Bilska, and D. A. Redmer. Uteroplacental vascular development and placental function: an update. Int. J. Dev. Biol. 2010 (in press).

Reynolds, L. P., P. P. Borowicz, K. A. Vonnahme, M. L. Johnson, A. T. Grazul-Bilska, J. M. Wallace, J. S. Caton, and D. A. Redmer. 2005b. Animal models of placental angiogenesis. Placenta 26:689-708.

Reynolds, L. P., P. P. Borowicz, K. A. Vonnahme, M. L. Johnson, A. T. Grazul-Bilska, J. M. Wallace, D. A. Redmer, and J. S. Caton. 2005c. Placental angiogenesis in sheep models of compromised pregnancy. J. Physiol. 565:43-58.

Reynolds, L. P., A. T. Grazul-Bilska, and D. A. Redmer. 2002. Angiogenesis in the female reproductive organs: pathological implications. Int. J. Exp. Pathol. 83:151-163.

Reynolds, L. P., R. R. Magness, and S. P. Ford. 1984. Uterine blood flow during early pregnancy in ewes: interaction between the conceptus and the ovary bearing the corpus luteum. J. Anim. Sci. 58:423-429.

Reynolds, L. P., and D.A. Redmer. 1998. Expression of the angiogenic factors, basic fibroblast growth factor and vascular endothelial growth factor, in the ovary. J. Anim. Sci. 76:1671-1681.

Reynolds, L. P., and D. A. Redmer. 2001. Angiogenesis in the placenta. Biol. Reprod. 64:1033-1040.

Reynolds, L. P., and D. A. Redmer. 1992. Growth and microvascular development of the uterus during early pregnancy in ewes. Biol. Reprod. 47:698-708.

Reynolds, L.P., and D. A. Redmer. 1995. Utero-placental vascular development and placental function. J. Anim. Sci. 73:1839-1851.

Rowe, A.J., C. Wulff, and H. M. Fraser. 2004. Angiogenesis and microvascular development in the marmoset (Callithrix jacchus) endometrium during early pregnancy. Reproduction 128:107-116.

Sherer, D. M., and O. Abulafia. 2001. Angiogenesis during implantation, and placental and early embryonic development. Placenta 22:1-13.

Spencer, T. E., G. A. Johnson, F. W. Bazer, R. C. Burghardt, and M. Palmarini. 2007. Pregnancy recognition and conceptus implantation in domestic ruminants: roles of progesterone, interferons and endogenous retroviruses. Reprod. Fertil. Dev. 19:65-78.

Spencer, T. E., G. A. Johnson, R. C. Burghardt, and F. W. Bazer. 2004. Progesterone and placental hormone actions on the uterus: insights from domestic animals. Biol. Reprod. 71:2-10.

Spencer, T. E., O. Sandra, and E. Wolf. 2008. Genes involved in conceptus-endometrial interactions in ruminants: insights from reductionism and thoughts on holistic approaches. Reproduction 135:165-179.

Stillerman, K. P., D. R. Mattison, L. C. Giudice, and T. J. Woodruff. 2008. Environmental exposures and adverse pregnancy outcomes: a review of the science. Reprod. Sci. 15:631-650.

Torry, D. S., M. Hinrichs, and R. J. Torry. 2004. Determinants of placental vascularity. Am. J. Reprod. Immunol. 51:257-268.

Wallace, J. M., D. A. Bourke, R. P. Aitken, N. Leitch, and W. W. Hay. 2002. Blood flows and nutrient uptakes in growth-restricted pregnancies induced by overnourishing adolescent sheep. Am. J. Physiol. – Reg. Integr. Comp. Physiol. 282:R1027-R1036.

Weems, C.W., Y. S. Weems, and R. D. Randel. 2006. Prostaglandins and reproduction in female farm animals. Vet. J. 171:206-228.

Wulff, C., M. Weigand, R. Kreienberg, and H. M. Fraser. 2003. Angiogenesis during primate placentation in health and disease.

Zheng, J., M. L. Johnson, D. A. Redmer, and L. P. Reynolds. 1996. Estrogen and progesterone receptors, cell proliferation, and c-fos expression in the ovine uterus during early pregnancy. Endocrinology 137:340-348. Reproduction 126:569-577.

NDSU Extension Service Live Lamb Carcass Contest Report

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Using ultrasound measurements for live carcass evaluation can be used as an effective tool for selection of carcass merit improvement. It has been used very little on sheep; however, the technology has been available to North Dakota youth participating in the sheep project.

Introduction

The use of ultrasound has been around for research purposes but not used as often in performance measurements for live evaluation until the last decade. In sheep it is used even less, but offers the same amount of accuracy as other species. Combining carcass traits with economic traits of importance such as growth, maternal traits, pedigree, and reproduction can make flock selection decisions for genetic improvement easier. The points evaluated for decision making purposes are ribeye area (REA), fat thickness (FT), and body wall thickness (BWT). These carcass traits are highly heritable and can be useful in determining extremes.

REA is measured in square inches between the 12-13th rib. It is positively correlated with carcass cutability, giving a good indicator of total muscling. REA reflects the differences in the proportion of muscle-to-bone within the carcass, and usually measures between 1.5 - 4.0 square inches. Fat thickness or backfat, is measured over the center of the ribeye at the 12 - 13th rib. The fat usually ranges from 0.1 - 0.5 inches. Fat thickness is the most important measurement that helps determine carcass cutability. As fat thickness increases, the percent BCTRC will decrease. Body wall thickness is a measurement across the lean, bone, and fat of the loser rib. This area can accumulate excess fat and thus, serves as an indicator of lean meat yield. BWT usually ranges from 0.5 - 1.2 inches.

Procedures

In the youth lamb project, the market lamb portion is one that allows smaller youth to get involved at a young age because the size of the animal may fit the size of the child, however that doesn't limit the knowledge of the participants. This project was started to allow youth that do not traditionally get to evaluate their carcasses a live glimpse at them. This can also help them to make their own decisions regarding technology such as ultrasound. This report marks the 2nd year of an ongoing project known as the North Dakota Live Lamb Carcass

Contest. In the sheep project for 4-H, FFA, or Junior sheep members, youth can enter their live market sheep for ultrasound measurements, weight measurements, and then combine that for an index of percent boneless closely trimmed retail cuts (% BCTRC=49.936-(0.0848 X HCW)-(4.376 X FT)-(3.530 X BW)+(2.456 X REA). This contest was offered at the North Dakota State Fair and was open to youth members. Weights and ultrasound measurements were taken from 109 lambs entered our database.

Results and Discussion

After the calculations for % BCTRC were determined the top 20 in the contest received awards from the North Dakota Lamb and Wool Producers Association. The top lamb % BCTRC was 50.63, had a 4.87 in. REA, and 0.5 in. FT. The range for all of the competitors was 2.13 - 5.40, BWT ranged from 0.47 - 1.77, FT ranged from 0.2 - 1.40, and the % BCTRC ranged from 43.84 - 50.63. Many of the ranges were larger than the previous year. This may be due to weather factors over this strange weather pattern year. There was a growing interest as well in the project. Many of the youth remembered this from last year and compared their previous results, but also analyzed all of the carcass factors.

Implications

With this ongoing project, we will be able to evaluate the progress of the youth market lamb project and how selection can affect carcass traits.

FLOCK CALENDAR OUTLINE

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

PRIOR TO BREEDING

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

BREEDING

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

PRIOR TO LAMBING (First 15 weeks)

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

LAST SIX WEEKS BEFORE LAMBING

1. Trim hooves and treat for internal parasites.
2. Six to four weeks before lambing feed 1/4 to 1/3 pound grain/ewe/day.
3. Shear ewe before lambing (with highly prolific ewes at least a month before is preferred). Keep feeding schedule regular and watch weather conditions immediately after shearing (cold).
4. Vaccinate ewe for enterotoxaemia.

5. Control lice and ticks immediately after shearing.
6. Four weeks before lambing increase grain to 1/2 to 3/4 pound/ewe/day (usually done immediately after shearing.
7. Give A-D-E preparations to ewes if pastures and/or roughage are or have been poor quality.
8. Feed selenium-vitamin E or use an injectable product if white muscle is a problem. Caution DO NOT use both.
9. Check facilities and equipment to be sure everything is ready for lambing.
10. Two weeks before lambing increase grain to 1 pound/ewe/day.

LAMBING

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

END OF LAMBING TO WEANING

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

WEANING

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

WEANING TO PRE-BREEDING

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

REARING LAMBS ARTIFICIALLY (ORPHANS)- MANAGEMENT TIPS

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

- It is essential that newborn lambs receive colostrums milk. Cow's colostrums will work if ewe's milk is not available. Do not dilute with water or warm too quickly if colostrums is frozen.
- Lambs should be removed from sight and hearing distance of ewes.
- Provide a warm, dry , draft-free area to start lambs.
- Use a good milk replacer that is 30% fat and at least 24% protein. Each lamb will require from 15 to 20 pounds of replacer to weaning.
- Lambs may require some assistance the first day or two to teach them to nurse on whatever feeding device is used.
- Start on nurser quickly, young lambs start easier.
- Self feed cold milk replacer after lambs are started. Milk replacers should be mixed with warm water for best results and then cooled down. Lambs feed cold milk well with less problems from scours and other digestive distrubances. Cold milk keeps better too.
- There is a Formaldehyde solution commercially avaliable that retards bacterial growth in milk (1cc/gallon milk).
- Hang a light over the milk replacer feeding device and dry ration feeder.
- Avoid placing young lambs with older lambs, as they may be pushed aside and may not be able to obtain the milk replacer. Remember that lambs nursing ewes drink 25 to 40 times per 24 hours. Best results have been obtained when lambs are fed in groups of 3 to 4 initially. After lambs are successfully trained, they can be handled in groups of 25.
- Inject lambs in the first few days with Iron Dextran, Vitamin A-D-E, and Selenium-Vitamin E. At 15 days of age, vaccinate for overeating (Colostridum perfringen type C & D).
- Provide lambs with a high-quality creep feed as soon as possible. Provide ample fresh water in front of lambs at all times. Do not feed hay or oats the first three week after weaning, as it encourages bloat. Caution! Do not feed leafy alfalfa until two weeks after weaning, as it encourages bloat.
- Wean lambs abruptly at 21-30 days of age. When to wean depends upon whether lambs are eating creep feed and drinking water. Newly weaned lambs will go back wards for several days. Don't be alarmed, they will make compensating gains later on.