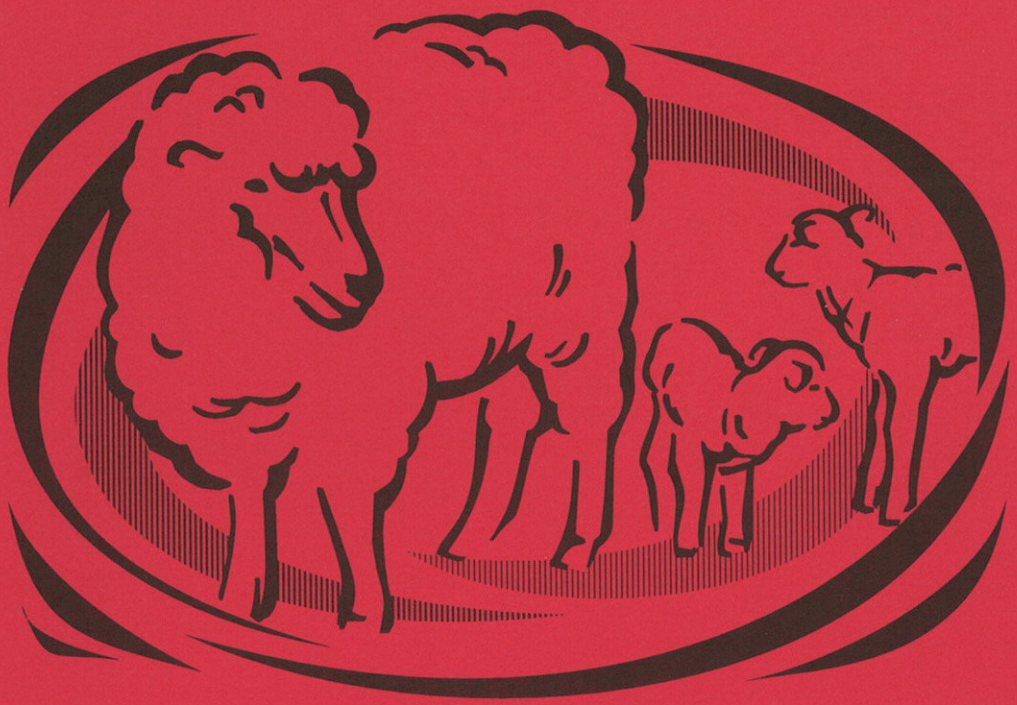
REPORT NO. 43



WESTERN DAKOTA SHEEP DAY

February 13, 2002

HETTINGER ARMORY

Hettinger Research Extension Center
and
Department of Animal and Range Sciences
North Dakota State University

February 13, 2002

Dear Sheep Producer:

On behalf of the Hettinger Research Extension Center and the Department of Animal and Range Sciences, let us welcome you to "Sheep Day". This report collectively represents North Dakota State University's efforts at both locations to provide information for the support of the sheep industry. We welcome your comments as grassroots users of the efforts of both Extension and Experiment Station resources. Your constructive comments assist us to participate meaningfully in the future of your industry.

A collective, positive and participatory attitude by producers and caretakers of their land grant resources will go far to solve problems confronting the sheep industry.

Best wishes for a day of sharing and learning.

Timothy C. Faller
Director
Hettinger Research Extension Center
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Chair
Dept. of Animal & Range Sciences
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PROGRAM

9:30 AM	**DOORS OPEN** - and coffee at Hettinger Armory (Adams County Farmers Union)
10:10 AM	Early Bird Door Prize Drawing (for Columbia ewe lamb)
10:15 AM	**HETTINGER AND FARGO REPORTS** Justin Luther (Physiology Studies) Marc Bauer, Hayley Encinias (Nutritional Studies) Jack Dahl, Luke Samuel, and Mitch Faulkner (Grazing Studies) Tim Faller (Out-of-Season Lambing) (New Project Previews)
11:45 AM	**"LASER SCAN DEMONSTRATION"** Dr. Rodney Kott, Sheep Specialist, Montana State University (Producer's wool samples will be tested free)
12:00 PM	**LUNCH: AMERICAN LAMB DINNER**
1:00 PM	**WELCOME:** Dr. James Venette North Dakota State University
1:10 PM	**"ANIMAL DAMAGE CONTROL HIGHLIGHTS"** Phil Mastrangelo, USDA/APHIS, Bismarck, North Dakota
1:25 PM	**"SUMMARY OF FALL LAMBING RESEARCH AT SDSU"** Lowell Slyter, SDSU, Brookings, South Dakota
2:05 PM	**"THE CHANGING FACE OF THE NORTH DAKOTA SHEEP INDUSTRY"** Roger Haugen, NDSU, Fargo, North Dakota
2:25 PM	**"A REALISTIC VIEW OF FALL LAMBING"** Jeff Held, SDSU, Brookings, South Dakota
2:55 PM	**"HOW SCRAPIE WILL EFFECT THE SHEEP INDUSTRY"** Susan Keller, North Dakota Department of Animal Health
3:25 PM	**"CLOSING COMMENTS"** Burton Pfliger, President North Dakota Lamb and Wool Producers Association

SHEEP DAY DIGEST

by
Timothy C. Faller, Director
Hettinger Research Extension Center
North Dakota State University

SECTION I
REPORTS OF RESEARCH IN PROGRESS
AT THE
HETTINGER RESEARCH EXTENSION CENTER
AND MAIN STATION
NORTH DAKOTA STATE UNIVERSITY

AT THE
43RD ANNUAL SHEEP DAY

HETTINGER RESEARCH EXTENSION CENTER
HETTINGER, NORTH DAKOTA

FEBRUARY 13, 2002

ND 1709 Objective: 1

OUT OF SEASON REPRODUCTIVE POTENTIAL OF WESTERN WHITE FACED RAMBOUILLET TYPE SHEEP UNDER NORTH DAKOTA CONDITIONS

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Introduction

The seasonal fertility of sheep continues to be a biological puzzle. Unlocking the puzzle offers much opportunity to the sheep industry. Many earlier studies indicate acceptable levels of success in getting sheep to conceive and lamb in non-traditional seasons, however, it usually has involved light control and or hormonal therapy. Many times there still has been some level of failure. Occasionally the level of management employed has confused the level of success of or predictability of out of season lambing schemes. The inability of sheep to consistently lamb according to chosen season severely restricts the development of a constant, dependable supply of lamb meat to consumers. If sheep were able to conceive consistently in April and subsequently lamb in mid to late September it would reduce necessity of quality facilities to maintain a breeding sheep operation under North Dakota climatic conditions . This production scheme would open opportunity to the most economically attractive markets for North Dakota producers as well. Similarly mature ewes involved in a fall lambing scheme would be available as leafy spurge grazers during typical summer months without the presence of lambs to reduce potential of predation. This would be extremely attractive insight of the level of problems associated with the presence of leafy spurge in North Dakota.

Procedure

Starting in 1986, Rambouillet ewes were randomly mated to Rambouillet rams and evaluated in a lambing system that anticipated the ewes to lamb three times in a two year period. In the spring of 1992 the flock was closed and the ewes were being evaluated based on the anticipation of breeding in April with a July clean up mating. The ewes were exposed each time with a 51 day breeding period starting April 4 and July 15. Ram to ewe ratios were one ram to twenty ewes. This closed flock was able to maintain consistent breeding success in April of 80-90 percent of the mature ewes. Replacement ewes were selected randomly from the September born ewe lambs similar to the selection of replacement rams. Poor growth or structurally incorrect individuals were removed from the population prior to making random selections. A control set of similar background ewes mated in November for April lambing has been maintained for the duration of the trials. Replacement ewes were exposed their first time in July along with the mature ewe flock and then re-exposed the following April regardless if they had conceived in the previous July. Ewes that did not maintain a lambing sequence that included every twelve month period starting with their first anticipated lambing time were eliminated from the flock.

In the fall of 1997 one hundred May born ewe lambs, of similar wool grade and structural size, were selected from a commercial sheep operation in Wyoming. The purpose was to compare breeding success when subjected to the exact same breeding strategy as the one hundred ewe

lambs selected from the September born closed flock ewes. Similar selections were made in the fall of 1998 and 1999 with the same intent. Rams from outside flocks were also purchased each year to service a 2x2 factorial design that included closed flock ewes mated to closed flock rams, closed flock ewes mated to purchased rams, purchased ewes mated to closed flock rams and purchased ewes mated to purchased rams. Ram to ewe ratios was maintained to be similar for all breeding groups. All ewes included in the project will be weighed and condition scored annually in the month of April. A five point condition scoring system will be employed with 1 being emaciated and 5 being obese. Routine performance measures will be recorded for the duration of the studies. A strict regimen of isolation of ewes from rams will be maintained other than during the desired mating periods to take advantage of any positive effects of the presence of the ram in enhancing the onset of estrus. Similar data will be collected for the original closed ewe flock that originated in 1986.

Results and Discussion

(Progress Report)

Table 1 indicates performance of the mature brood ewe flock that has been maintained as a closed fall lambing flock since 1986. All ewes were exposed to mate in April with clean-up mating in July-August. Table 1 indicates success of mating naturally without light control or hormonal therapy. Success would be categorized to be quite similar to traditional fall mating for spring lambing.

Table 1. Mature flock lambing performance for 1999 and 2000.

Ewe Birth Year	**1993**	**1994**		**1995**		**1996**	
Lambing Season	1999	1999	2000	1999	2000	1999	2000
Ewe Exposed	55	56	42	62	51	96	84
Ewes Lambing	55	53	38	60	50	87	81
Percent Bred to Fall	100	95	90	97	98	91	96

Table 2 and 3 indicates ewe body condition scores and mean weights for ewes exposed to lamb their first time in the fall. These measures would represent purchased ewes at 22 months of age and those from the closed flock being 17 months of age at breeding time in April. The data would indicate that the purchased ewes perform very similar to the ewe flock that has been selected for fall lambing.

Table 2. Body condition score and percentage of ewes in the condition score categories going into the 1999, 2000, and 2001 breeding season.

	1999	2000		2001		
Ewe Birth Year	1997	1997	1998	1997	1998	1999
Closed Ewes X Closed Ram						
Condition Score & % of Ewes	2=34% 3=66%	2=22% 3=78%	2=55% 3=45%	2=09% 3=82% 4=09%	2=24% 3=76%	2=79% 3=21%
Closed Ewes X Purch Ram						
Condition Score & % of Ewes	1=02% 2=31% 3=67%	2=29% 3=68% 4=04%	2=50% 3=45% 4=05%	2=09% 3=91%	2=24% 3=76%	2=47% 3=53%
Purch Ewes X Closed Ram						
Condition Score & % of Ewes	2=04% 3=62% 4=04%	2=06% 3=91% 4=03%	2=24% 3=76%	3=92% 4=08%	2=03% 3=97%	2=33% 3=67%
Purch Ewes X Purch Ram						
Condition Score & % of Ewes	2=27% 3=73%	2=08% 3=92%	2=08% 3=92%	2=11% 3=75% 4=14%	2=05% 3=95%	2=40% 3=60%

Table 3. Mean weight of ewes going into the 1999, 2000, and 2001 breeding season.

	1999	2000		2001		
Ewe Birth Year	1997	1997	1998	1997	1998	1999
Closed Ewes X Closed Ram						
lbs (Standard Error)	112 (2.5)	137 (2.8)	111 (2.8)	145 (3.3)	131 (2.3)	103 (2.2)
Closed Ewes X Purch Ram						
lbs (Standard Error)	115 (3.1)	141 (3.7)	113 (3.1)	144 (2.7)	124 (2.5)	109 (3.9)
Purch Ewes X Closed Ram						
lbs (Standard Error)	117 (2.8)	143 (3.3)	120 (3.2)	153 (2.8)	138 (2.5)	125 (2.6)
Purch Ewes X Purch Ram						
lbs (Standard Error)	111 (2.0)	145 (2.9)	122 (3.6)	149 (3.9)	136 (2.2)	120 (2.5)

Table 4 compares mean weights for ewes going into the breeding season as two year olds. Results showed that were no differences ($P>0.05$) among 1997, 1998, and 1999 class ewes in the closed ewes and closed ram (CECR), and the closed ewes and purchased ram (CEPR) treatment as two year olds. In both the purchased ewes and closed ram (PECR) and purchased ewes, and purchased ram (PEPR) treatments there were differences ($P\leq0.05$) in mean weights as

two year olds going into the breeding season. Results also showed that in both the 1998 and 1999 class ewes, as two year olds, in the PECR and PEPR were significantly higher ($P \leq 0.05$) in mean weights going into the breeding season than the CECR and CEPR treatments. In both the PECR and PEPR the 1999 class ewes were significantly higher ($P \leq 0.05$) than the 1997 class of ewes, as two year olds (Table 4).

Table 4. Mean weight of ewes as two year olds going into breeding season.

Ewe Birth Year	1997 [1]	1998 [1]	1999 [1]
Closed Ewes X Closed Ram [2]			
lbs (Standard Error)	112 (2.5)^{ax}	111 (2.8)^{ax}	103 (2.2)^{ax}
Closed Ewes X Purch Ram [2]			
lbs (Standard Error)	115 (3.1)^{ax}	113 (3.1)^{ax}	109 (3.1)^{ax}
Purch Ewes X Closed Ram [2]			
lbs (Standard Error)	117 (2.8)^{ax}	120 (3.2)^{aby}	125 (2.6)^{by}
Purch Ewes X Purch Ram [2]			
lbs (Standard Error)	111 (2.0)^{ax}	122 (3.6)^{by}	120 (2.5)^{by}

[1] Mean weights within the ewe birth year with the same letter are not significantly different (P>0.05) (a and b).
[2] Mean weights within the same treatment with the same letter are not significantly different (P>0.05) (x and y).

Table 5 shows mean weights of ewes as three year olds going into the breeding season. There were no differences (P>0.05) among treatments in the 1997 class ewes going into the breeding season as three year olds. Differences were found among treatments in the 1998 class ewes going into the breeding season. The CEPR treatment was significantly ($P \leq 0.05$) lower in mean weights than the PECR and PEPR treatments going into the breeding season. Results also showed that there were in differences ($P \leq 0.05$) among the 1997 and 1998 class ewes going into the breeding season as three year olds. The 1997 class ewes in the CEPR and PEPR treatments had a higher ($P \leq 0.05$) mean weight going into the breeding season (Table 5).

Table 5. Mean weight of ewes as three year olds going into breeding season.

Ewe Birth Year	1997[1]	1998[1]
Closed Ewes X Closed Ram[2]		
lbs (Standard Error)	137 (2.8)^{ax}	131 (2.3)^{abx}
Closed Ewes X Purch Ram[2]		
lbs (Standard Error)	141 (3.7)^{ax}	124 (2.5)^{ay}
Purch Ewes X Closed Ram[2]		
lbs (Standard Error)	143 (3.3)^{ax}	138 (2.5)^{bx}
Purch Ewes X Purch Ram[2]		
lbs (Standard Error)	145 (2.9)^{ax}	136 (2.2)^{by}

[1] Mean weights within the ewe birth year with the same letter are not significantly different (P>0.05) (a and b).
[2] Mean weights within the same treatment with the same letter are not significantly different (P>0.05) (x and y).

Table 6 indicates reproductive performance of the four breeding schemes described in the procedure. Numbers of ewes available at time of breeding were reduced from the original one hundred closed flock ewes and one hundred purchased ewes because of predation, loss of ear tags and other natural causes. Early indications are that the purchased ewes and rams performed at a level higher than anticipated for first exposure for fall lambing. Initially there appeared to be a positive influence when using closed flock rams on purchased ewes, this effect diminished in the second year of production.

Table 6. Fall Lambing Performance of Purchased versus Closed Flocks during the 1999, 2000, and 2001 lambing season.

	1999	**2000**		**2001**				
Ewe Birth Year	1997	1997	1998	1997		1998		1999
Closed Ewes X Closed Ram								
Ewes Exposed	42	38	35	32		36		31
Ewes Pregnant	33	30	34	29		35		12
Fall Breeding %	79%	79%	97%	91%	***83%[1]***	97%	***97%[1]***	39%
Closed Ewes X Purch Ram								
Ewes Exposed	43	40	31	29		33		31
Ewes Pregnant	28	32	27	26		30		15
Fall Breeding %	65%	80%	87%	90%	***78%[1]***	91%	***89%[1]***	48%
Purch Ewes X Closed Ram								
Ewes Exposed	43	37	34	32		29		38
Ewes Pregnant	33	31	31	29		26		31
Fall Breeding %	72%	84%	91%	91%	***82%[1]***	90%	***91%[1]***	82%
Purch Ewes X Purch Ram								
Ewes Exposed	44	39	35	32		34		36
Ewes Pregnant	23	31	33	30		26		22
Fall Breeding %	52%	79%	94%	94%	***75%[1]***	76%	***85%[1]***	61%

[1]***Indicates a percentage of fall breeding over three breeding seasons for the 1997 class ewes and two breeding season for the 1998 class ewes.***

Summary

This being the second year of a multiple year trial no attempt was made to analyze the data for differences. It will be especially important to evaluate year two through four and to see if the purchased ewes breeding performance improves at similar rates as closed flock individuals as they mature in the system. They will continue to be measured as a comparison to the base closed flock.

EFFECTS OF MULTI-SPECIES GRAZING ON LEAFY SPURGE (*Euphorbia esula* L.) INFESTED RANGELAND USING ROTATIONAL GRAZING
(A Four-Year Summary)

[1]Luke W. Samuel, [1]Kevin K. Sedivec, [2]Timothy C. Faller,
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Introduction
Leafy spurge is a plant widely dispersed across the northern hemisphere, including the United States and Canada, with a distribution center in the Caucasus Region of Asia (Croizat 1945 in Noble and MacIntyre 1979). This plant is found on every continent except Australia (Lacey et al. 1985). Leafy spurge is believed to have been introduced into mainland North America before 1872 (Callihan et al. 1991). Leafy spurge now infests thirty-nine states in the United States including every northern state and every Canadian province except Newfoundland (Lacey et al. 1985). One million hectares (2.5 million acres) in North America are infested by leafy spurge (Noble et al. 1979 in Noble and MacIntyre 1979), with an estimated 400,000 hectares (one million acres) in North Dakota (N.D. Dept. of Agriculture 1996).

Traditional approaches for controlling leafy spurge, e.g. herbicides, are becoming cost prohibitive as this noxious weed continues to spread. Many forms of biological and cultural controls have come into practice over the past twenty years. Grazing sheep on leafy spurge infested rangeland is one such cultural control. Cattle do not graze leafy spurge and often avoid leafy spurge-infested communities, creating opportunities for multi-species grazing with sheep. Multi-species grazing is the concurrent use of rangeland by more than one kind of animal, and this approach utilizes more than one class of vegetation (Merrill et al. 1966). Cattle and sheep grazing has the potential to reduce leafy spurge density, increase plant species richness, and improve the economic viability of a cattle operation on leafy spurge infested rangelands.

Research Objectives
The objectives of the study were to determine if simultaneous grazing of leafy spurge infested rangeland with cattle and sheep employing a twice-over-rotational grazing system in conjunction with biological control (insects) will (1) reduce leafy spurge density compared to season-long grazing and (2) enhance livestock grazing efficiency compared to season-long grazing.

Study Area and Design
This project was conducted on leafy spurge infested rangeland in western North Dakota from 1998 through 2001. The study area is located approximately ten kilometers (six miles) north of Sentinel Butte or 240 kilometers (150 miles) west of Bismarck, North Dakota. Two tracts of rangeland of

257 and 160 hectares (635 and 395 acres) comprise the replicated multi-species grazing trial in the Badlands vegetative region of North Dakota. Vegetation in this region is typical of northern mixed grass prairie and is classified as a wheatgrass-grama-needlegrass (*Agropyron, Bouteloua, Stipa*) plant community (Barker and Whitman 1989). Leafy spurge infested approximately forty to fifty percent of the land on these two study sites.

This trial was designed to test the effects of twice-over rotation (TOR) and season-long (SL) grazing on leafy spurge infested rangeland using multi-species grazing with cattle and sheep in combination with a biological control program. Each of two tracts of land were blocked into four cells with one cell randomly selected as a SL treatment. The remaining three cells in each replicate were grazed using TOR grazing treatment. Two 0.40 hectare (one acre) exclosures were developed on each replicate by stratifying each treatment by upland and lowland sites and randomly selecting points for development. The four exclosures, containing forty to fifty percent leafy spurge, were excluded from grazing and classified as biological control (insect) treatments.

Fifty permanent 100-meter line transects were systematically located in leafy spurge communities (26 transects) and native range (devoid of leafy spurge) vegetation sites (24 transects) on both replicates to monitor changes in leafy spurge stem density and plant species richness. Barbour et al. (1999) defined density as the number of plants rooted within each quadrat. Species richness is simply the number of species per unit area; diversity is a combination of richness and evenness, i.e., species richness weighted by species evenness (Barbour et al. 1999). Peet (1974 in Ludwig and Reynolds 1988) termed this "the dual concept of diversity," i.e., diversity combines species richness and relative species abundance.

Four transects were located in each cell of the TOR grazing treatments, eight transects in each SL treatment, and two in each of the biological control cells. In addition, two permanent line transects designed to monitor effects of leafy spurge on rangeland without grazing, biological, or other management were located in areas dominated by leafy spurge adjacent to each replicate.

Leafy spurge density and graminoid species frequency was collected every five meters using a 0.10 square meter frame and forb and shrub density and frequency was collected every five meters using a 0.25 square meter frame on the 100-meter line transects.

Livestock performance and production data was collected for cattle and sheep by determining average daily weight gain and gain per area. Livestock were weighed at the start and end of each grazing season.

Treatment and year effects for leafy spurge stem density and livestock performance were analyzed using a general linear model and univariate analysis (GLM) (SPSS 1999). A mean separation was performed when significant ($P<0.05$) differences were found using Tukey's Honesty Significant Difference (SPSS 1999).

Grazing Treatments and Grazing Plan

Cattle grazed each treatment from 1 June through 15 September while stocked in accordance with the recommended carrying capacity of the land as outlined in USDA Natural Resources

Conservation Service Technical Guidelines (1984). Sheep grazed from 15 May through 15 September and stocked at forty percent of the original carrying capacity without adjustments to cattle numbers.

Carrying capacity of the TOR grazing treatment was 142.4 animal unit months (AUMs) and 73.6 AUMs on replicates #1 and #2, respectively. Stocking rates of the TOR grazing treatments were 0.28 AUMs/acre for both replicates #1 and #2. Type of cattle grazed was Angus-Hereford cross cow/calf pairs with cows weighing approximately 545 kilograms (1200 pounds). Thirty-six cow/calf pairs grazed replicate #1 and 18 cow/calf pairs grazed replicate #2. Since sheep were stocked at forty percent of the carrying capacity, sheep were grazed at 57.5 AUMs (replicate #1) and 33 AUMs (replicate #2) on the TOR grazing treatments. Type of sheep were mature white-faced ewes of which 86 head grazed on replicate #1 and 45 head grazed on replicate #2.

Carrying capacity of the SL grazing treatment was 39.6 and 33.9 AUMs on replicates #1 and #2, respectively. Stocking rates of the SL grazing treatments were 0.31 and 0.32 AUMs/acre on replicates #1 and #2, respectively. Type of cattle grazed was Angus-Hereford cross cow/calf pairs with cows weighing approximately 545 kilograms (1200 pounds). Ten cow/calf pairs grazed replicate #1 while 8 cow/calf pairs grazed replicate #2. Since sheep were stocked at forty percent of the carrying capacity, sheep were grazed at 16 AUMs (replicate #1) and 15 AUMs (replicate #2) on the SL grazing treatments. Type of sheep grazed was mature white-faced ewes, with 23 head on replicate #1 and 20 head on replicate #2.

Livestock graze the SL treatment continuously throughout the grazing season. Livestock graze the TOR grazing treatment as one herd and rotate simultaneously. The entire herd of cattle and sheep graze one cell at a time, grazing forty percent of the available carrying capacity of the cell in the first rotation and sixty percent of available carrying capacity in the second rotation.

Results and Discussion

After four grazing seasons, leafy spurge stem densities were significantly ($P<0.05$) reduced on both grazing treatments and bio-control treatment on lowland and upland leafy spurge sites (Table 1 and 2). There were no ($P>0.05$) differences between the SL, TOR or non grazed bio-control leafy spurge treatments. The lowland sites on the TOR grazing treatment had the greatest decrease in leafy spurge stem densities at 80%. The SL treatment achieved the best control on upland sites with a decrease of 72%

Table 1. Leafy spurge stem densities in the lowland sites on the bio-control only, season long (SL) and twice-over-rotation (TOR) grazing treatments in 1998 and 2001.

Treatment[1]	1998[2]	2001[2]	% Decrease from 1998 to 2001
	# Stems/ 0.10 m^2		
Bio-Control	13.9 ± 1.3^{ax}	3.1 ± 0.6^{ay}	78%
Season-long	18.4 ± 1.1^{ax}	6.7 ± 1.2^{ay}	63%
Twice-over	18.2 ± 0.8^{ax}	3.5 ± 0.6^{ay}	80%

[1] **Treatments with the same letter are not significantly different (P≥0.05) (a, b, and c).**
[2] **Years with the same letter within each treatment are not significantly different (P≥0.05) (x, y, and z).**

Table 2. Leafy spurge stem densities in the upland sites on the bio-control only, season long (SL) and twice-over-rotation (TOR) grazing treatments in 1998 and 2001.

Treatment[1]	1998[2]	2001[2]	% Decrease from 1998 to 2001
	# Stems/ 0.10 m^2		
Bio-Control	8.8 ± 0.8^{ax}	3.3 ± 0.6^{ay}	63%
Season-long	9.7 ± 0.8^{ax}	2.7 ± 0.4^{az}	72%
Twice-over	9.1 ± 0.6^{ax}	3.0 ± 0.3^{ay}	67%

[1] **Treatments with the same letter are not significantly different (P≥0.05) (a, b, and c).**
[2] **Years with the same letter within each treatment are not significantly different (P≥0.05) (x, y, and z).**

Cow average daily gain (ADG) was not (P>0.05) different between TOR and SL treatments during the four years of the study. However, cow ADG was lower (P<0.05) in 1999 compared to 1998, 2000 and 2001 on the TOR and SL treatments. Calf ADG also did not differ (P>0.05) between TOR and SL treatments for all four years. However, Calf ADG was lower (P<0.05) in 1998 then in 2000 on the SL treatment (Table 3).

There was no (P>0.05) difference in ewe ADG between TOR and SL treatments over the four years of the study. Ewe ADG was higher (P<0.05) on SL and TOR treatments in 1999 compared to 1998, 2000 and 2001. Ewe ADG on the TOR was also higher in 2001 than in 1998 (Table 4).

Table 3. Cow and calf average daily gains (ADG) for the season long (SL) and twice-over-rotation (TOR) treatments from 1998, 1999, 2000 and 2001.

Treatment		1998	1999	2000	2001
		lb/day			
Season-long	**Cows**	$1.13 \pm .12^{ax}$	$0.01 \pm .14^{ay}$	$1.21 \pm .13^{ax}$	$1.02 \pm .20^{ax}$
	Calves	$2.23 \pm .08^{axy}$	$2.28 \pm .08^{axy}$	$2.43 \pm .06^{ax}$	$2.72 \pm .07^{axz}$
Twice-over	**Cows**	$0.80 \pm .08^{ax}$	$0.07 \pm .07^{ay}$	$0.78 \pm .10^{ax}$	$1.15 \pm .20^{ax}$
	Calves	$1.99 \pm .05^{ax}$	$2.19 \pm .07^{axy}$	$2.22 \pm .04^{ay}$	$2.46 \pm .05^{az}$

[1] Treatments with the same letter for the same class of livestock are not significantly different (P≥0.05) (a, b, and c).

[2] Years with the same letter for the same class of livestock within each treatment are not significantly different (P≥0.05) (x, y, and z).

Table 4. Ewe average daily gains (ADG) for season long (SL) and twice-over-rotation (TOR) treatments during the 1998, 1999, and 2000 grazing seasons.

Treatment[1]	1998[2]	1999[2]	2000[2]	2001[2]
	lb/day			
Season long	$0.21 \pm .01^{ax}$	$0.35 \pm .02^{ay}$	$0.26 \pm .02^{az}$	$0.24 \pm .02^{axz}$
Twice-over	$0.20 \pm .003^{ax}$	$0.36 \pm .009^{ay}$	$0.25 \pm .01^{axz}$	$0.25 \pm .008^{az}$

[1] Treatments with the same letter are not significantly different (P≥0.05) (a, b, and c).

[2] Years with the same letter within each treatment are not significantly different (P≥0.05) (x, y, and z).

Summary

The preliminary results from this trial are encouraging. The addition of sheep to a cattle only grazing operation was shown to effectively reduce leafy spurge stem densities. To date, both the SL and TOR treatments have performed well in the control of leafy spurge over the four years of the study, with both SL and TOR grazing treatments significantly reducing leafy spurge stem densities. However, leafy spurge stem densities in the non grazed bio control exclosures were also reduced, and was not different than the reduction seen in the grazing treatments. This could be due to the fact that the bio-control exclosures were located inside the grazing study and comprised only 0.4% of the total area of the study, with the bio-control agents benefiting from the grazing treatments. Bio-control and grazing were both proven to be effective in reducing leafy spurge stem densities. However, the use of sheep as a long term management tool of leafy spurge without bio-control is still a proven method of control (Dahl et al. 2001) that allows producers to utilize available resources while providing added income. The use of bio-control does not provide the additional income that

can accompany the production of sheep, but costs and labor factors may prevent the use of sheep as a control method. Multi-species grazing and bio-control both provide effective control of leafy spurge, and a land owner can implement a project using one or both techniques that best meets their needs.

Overall, cattle and sheep grazing simultaneously did not adversely affect the ADG of cows, calves, or ewes using either grazing treatment. Multi-species grazing is a good alternative for leafy spurge control while allowing for an increased carrying capacity of the land with no adverse affects on livestock performance.

Literature Cited

Barbour, M.G., J.H. Burk, W.D. Pitts, F.S. Gillam, and M.W. Schwartz. 1999. Terrestrial plant ecology. Addison Wesley Longman, Inc., Menlo Park, Calif.

Barker, W.T. and W.C. Whitman. 1989. Vegetation of the northern Great Plains. NDSU Research Rpt. No. 111. Fargo.

Callihan, R.H., J.P. McCaffrey, and V.J. Parker-Clark. 1991. Leafy spurge: biology and management. Univ. of Idaho College of Ag., Cooperative Ext. System Ag. Exp. Sta. Current Information Series 877.

Dahl, J.D., K.K. Sedivec, T.C. Faller, D. Stecher, J.F. Karn, P.E. Nyren, and L. Samuel. 2001. Effects of multi-species grazing and single species grazing on leafy spurge infested rangeland (A five-year summary). N.D. State Univ. Ext. Service Sheep Day publication.

Lacey, C.A., P.K. Fay, R.G. Lym, C.G. Messersmith, B. Maxwell, and H.R. Alley. 1985. The distribution, biology and control of leafy spurge. Montana State Univ. Cooperative Ext. Service Circular 309, in cooperation with NDSU and the Univ. of Wyoming.

Ludwig, J.A. and J.F. Reynolds. 1988. Statistical ecology: a primer on methods and computing. John Wiley and Sons, New York, New York.

Merrill, L.B., P.O. Reardon, and C.L. Leinweber. 1966. Cattle, sheep, goats... mix'em up for higher gains. Texas Agr. Prog. 12:13-14

Noble, D.L. and D.C. MacIntyre. 1979. Management program for leafy spurge. Rangelands 1:247.

North Dakota Dept. of Agriculture. 1996. Weed control survey by county. N. D. Dept. of Ag., State Capitol Building, Bismarck.

SPSS. 1999. Statistical producers fro Social Science. Marketing dept. SPSS. Inc. Chicago, Ill.

MULTI-SPECIES GRAZING AND SINGLE SPECIES GRAZING ON LEAFY SPURGE INFESTED RANGELAND (Six-Year Summary)

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Acknowledgments: We would like to give special and well-deserved thanks to the Hettinger Research Extension Center staff (Don Stecher, Dennis Bentsen, Dave Pearson, Rick Olson, and Terri Lindquist). Also we would like to thank Dennis Whitted and Connie O'Brien for their help in collecting vegetative data.

Introduction

The use of sheep as a biocontrol agent in the control of leafy spurge is not a new concept. In the late 30's and early 40's Christensen et al. (1938), Helgeson and Thompson (1939) and Helgeson and Longwell (1942) indicated that sheep consumed leafy spurge and should be integrated into management strategies in controlling leafy spurge, however, there was limited promotion. Herbicides continue to be the primary method for control of leafy spurge (Lym et al. 1995). Many areas infested with leafy spurge, however, are in environmentally sensitive areas and most herbicides for controlling leafy spurge are not labeled for application in these sensitive areas. Which have lead many land managers to choose an alternative control agent such as Angora goats or sheep. Research conducted in the 1980's and 1990's has shown that sheep or goats will reduce leafy spurge stem densities and increase grass and grass-like disappearance, and there are significant benefits in using multi-species grazing to manage leafy spurge infested rangelands (Prosser 1995).

Weedy forbs and brush cause more losses on the United States 252 million hectares of rangeland than all other pests combined (Quimby et al. 1991). Based on this report it amplifies the use of sheep or goats in the control of other troublesome forbs and brush as well as leafy spurge found on many rangelands that have been traditionally grazed by cattle only.

Multi-species grazing is an important idea in rangeland management because rangelands usually consist of one or more classes of vegetation (Merrill et al. 1966). By using more than one livestock species on a given rangeland containing various vegetative communities provides the potential of increasing red meat production, species diversity, vegetative production, and revenue for a given ranching operation, with proper management plans. Although multi-species grazing provides the above benefits, the introduction of leafy spurge and its consistency of infesting grasslands in the mid-west exploit the importance of using a multi-species grazing approach.

The objectives of this study were to test the effects of multi-species and single species grazing treatments using cattle and sheep on: 1) differences in leafy spurge control, plant species richness

and density, plant species diversity, 2) evaluate differences in utilization levels by plant type and herbage production, and 3) evaluate differences in livestock weight gain.

Study Area

This study was conducted on Section 32, T139N, R81W of Morton County owned by the North Dakota State Correction Center in south central North Dakota, approximately two miles southwest of Mandan, and on the north half of Section 9 T138N, R81W of Morton county on native rangeland operated by the Northern Great Plains Research Laboratory, approximately three miles south of Mandan. The study area was located in the Missouri Slope Prairie region. Vegetation in this region is typical of northern mixed grass prairie (Barker and Whitman 1989) and classified as a wheatgrass-grama-needlegrass (*Agropyron, Bouteloua, Stipa*) plant community (Shiflet 1994).

Grazing treatments were multi-species and single species grazing on three replicated 20 acre blocks. Replicate one and two were within the North Dakota State Correction Center land and replicate three on the Northern Great Plains Research Laboratory. Each of the replicates were subdivided into 5 acre plots and treated with either a cattle only treatment (CO), sheep only treatment (SO), cattle and sheep treatment (CS) or a non use control (NU). Treatments were randomly selected within each block. The experimental design was a randomized complete block design (RCBD).

Sheep were placed on treatments approximately 15 May and cattle 1 June when native cool season grass species reach grazing readiness (3-4 leaf stage). Livestock species were removed from treatments when 50 to 60 percent degree of grass and grass-like species use or before 15 September.

Each replicated research block had one plot grazed by yearling steers (CO), one grazed by mature ewes (SO), and one grazed by yearling steers and mature ewes (CS). Stocking rates include two yearling steers for the CO from 1996 to 2001; twelve mature ewes in 1996, ten-mature ewes 1997 and 1998, and seven mature ewes from 1999 to 2001 for the SO; one yearling steer and six mature ewes in 1996 and one yearling steer and five mature ewes for the CS from 1997 to 2001. Stocking rates were about 1.5 AUMs/acre for the CO, SO, and CS treatments. Stocking rates for this trial were designed for 3.5 months of grazing for the steers and 4 months of grazing for the ewes. The flexible stocking rates on the SO and CS in sheep was due the adjustment in leafy spurge control and range condition.

Methods

Leafy spurge stem density counts were obtained by using a permanent 109.4 yard line transect and counts collected approximately every 5 ½ yards using a 1.08 ft^2 quadrat. One transect was systematically placed in each of the four treatments (CO, SO, CS, and NU) for each replicate. Transects were selected based on leafy spurge location within the treatments to assure full length of transect comprised leafy spurge. Leafy spurge densities were monitored over the five years to detect effectiveness of sheep grazing to control. Leafy spurge densities will be collected annually, around the end of May, for the duration of the study.

Forb and shrub species diversity and densities were determined using a 2.7 ft^2 quadrat. Nested within the 2.7 ft^2 quadrat was a 1.08 ft^2 quadrat used to determine grass and grass-like species diversity. Data was collected from 109.4 yard transects with readings conducted approximately every 5 ½ yards. Data was collected on all treatments and replicate from the leafy spurge transect developed to monitor leafy spurge stem density counts. One native (non-infested) 109.4 yard transect was located within each replicated treatment to monitor species diversity and density changes that may naturally occur due to treatment. Readings were collected from the native transects annually, except 1997. The leafy spurge transects were monitored annually and will continue to be monitored annually throughout the ten-year trial.

Leafy spurge, grass and grass-like, shrub, and forb herbage production was determined by clipping in late July on the NU treatment when vegetative species reached peak production (Whitman et al. 1952). The NU was stratified into 7.67 by 7.67 yard plot. A 7.67 yard buffer's strip was implemented to prevent edge effect. Twenty-five plots were randomly selected and clipped within each NU using a 2.7 ft^2 quadrat.

Degree of disappearance of leafy spurge, grass and grass-like, forbs, and shrubs were determined for each treatment at the end of the grazing season by stratifying each treatment into 7.67 by 7.67 yard quadrats in 1996, 1997, 1998, and 1999. Twenty-five quadrats were randomly selected and clipped using a 2.7 ft^2 quadrat for each grazed and non-use treatment to determine the degree of disappearance. The method of determining degree of disappearance was change in 2000 due to the change in herbage production on the grazing treatments. Degree of disappearance was monitored using the pair-plot technique, in 2000 and will continue throughout the duration of the trial, two frames within the cage and two out were clipped after the removal of livestock species. Five cages were systematically placed within each grazing treatment (CO, SO, and CS) in leafy spurge infested sites. This method allowed use to monitor the herbage production on the grazing treatments and the degree of disappearance of grass and grass-like, forbs, shrubs, and leafy spurge.

Livestock performance and production were collected for both cattle and sheep by determining average daily gain and gain per acre, respectively. Both classes of livestock were weighed prior to pasture turn out and monthly to follow performance throughout the grazing season. Final livestock weights were collected at the end of grazing season.

Treatment and year effects for leafy spurge stem density, forb and shrub density, herbage production, degree of disappearance, and livestock performances were analyzed using a general linear model (GLM) (SPSS 2000). A mean separation was performed using Tukey's Honesty Significant Difference when significant ($P \leq 0.05$) differences were found. The Shannon Wiener Index was used to calculate species diversity indices for both leafy spurge infested and non-infested range sites. Treatment and year effects of species diversity were analyzed using a non-parametric test (Krushal-Wallis Test) (SPSS 2000).

Results and Discussion

A significant ($P \leq 0.05$) reduction in leafy spurge stems occurred after one grazing season on the SO treatment and in three grazing seasons on the CS treatment. Leafy spurge was reduced from 10.4 stems/1.08 ft^2 in 1996 to 0.5/1.08 ft^2 stems in 2001, a reduction of 36% after one grazing

season and 95% after five on the SO. Leafy spurge stem densities were not affected after two grazing seasons on the CS treatment, however, by the third year the CS treatment had a significant ($P \leq 0.05$) reduction and results showed that a significant ($P \leq 0.05$) change between year three and four. Leafy spurge stems were reduced ($P \leq 0.05$) from 11.6 stems/1.08 ft^2 in 1996 to 1.2 stems/1.08 ft^2 in 2001, a reduction of 90% after five grazing seasons (Table 1).

Leafy spurge and non-infested range sites were significantly ($P \leq 0.05$) different in forb and shrub density on the NU treatment throughout five grazing seasons. Non-infested range sites had a higher ($P \leq 0.05$) forb and shrub density/2.7 ft^2 than leafy spurge range sites. Results after two grazing seasons showed that there were no differences ($P > 0.05$) between non-infested and leafy spurge range sites on the CO, SO, and CS grazing treatments in forb and shrub densities (Table 2). By the third year of grazing forb and shrub density on treatments CO and SO showed that there was no difference ($P > 0.05$) between the non-infested and leafy spurge range sites. Treatments, however, again showed significant differences ($P \leq 0.05$) between leafy spurge infested and non-infested sites in the forth and fifth grazing seasons.

Species diversity results showed that there were significant ($P \leq 0.05$) differences between leafy spurge and non-infested range sites in all treatments. In all of the treatments non-infested range sites were higher ($P \leq 0.05$) in species diversity than leafy spurge infested sites. Results also showed that species diversity did not change ($P > 0.05$) after five grazing seasons and there was no treatment or year effect present after the five years of grazing (Table 3).

Herbage production was different ($P \leq 0.05$) between growing seasons in grass and grass-like lb/acre. Results showed that graminoid grass and grass-like lb/acre was lower ($P \leq 0.05$) in 1998 than 1996, 1999 and 2000, however, was similar ($P > 0.05$) to 1997. Grass and grass-like production was higher ($P \leq 0.05$) than all growing seasons. Leafy spurge production was significantly higher ($P \leq 0.05$) in 2000 than 1998 and 2001, however, similar to production in 1996, 1997, and 1999 (Table 4).

Leafy spurge degree of disappearance increased on all sheep treatments from 1996 to 2001. The SO treatment went from 76% to 99% leafy spurge disappearance from 1996 to 2001, and the CS treatment went from 62% to 97% from 1996 to 2000. There was an increase ($P \leq 0.05$) in leafy spurge disappearance in the CO treatment with 23% disappearance in 1996 compared with 50% in 1997 and 1998; however, reduced again to 23% in 1999. These results in leafy purge disappearance on the CO treatment would suggest that steers were consuming leafy spurge; however, due to the design and location of watering facilities, the leafy spurge disappearance was more likely due to a trampling effect. As graminoid disappearance increased on CO treatment, so did leafy spurge disappearance, suggesting more use of the graminoids, more grazing and trampling occurs. Graminoid degree of disappearance was similar ($P > 0.05$) throughout the grazing seasons within and between grazing treatments for all years except 1999, where graminoid disappearance was reduced on the sheep treatments.

Steer average daily gain (ADG) was not different ($P > 0.05$) between treatments (CO and CS) after five grazing seasons of the study (Table 5). There was no change ($P > 0.05$) in steer ADG between years on the CO and CS treatment. Ewe ADG was not different ($P > 0.05$) between treatments (SO and CS) for either years of the study. There was a decrease ($P \leq 0.05$) in ewe ADG between years 1996 and 1998 on both SO and CS treatments, however, ADG was

significantly higher ($P \leq 0.05$) in 1999 than the 1998 grazing season (Table 5). These results would suggest multi-species grazing had no negative or positive impact on sheep or cattle performance compared with single species grazing.

Table 1. Leafy spurge stem densities on the cattle only (CO), sheep only (SO), cattle and sheep (CS), and control (NU) treatments from1996 through 2001. (SE in parentheses.)

	CO[2]	SO[2]	CS[2]	NU[2]
	# of Stems/1.08 ft^2 quadrat			
1996[1]	9.8 (1.2)^{abx}	10.4 (0.9)^{ax}	11.6 (1.0)^{ax}	9.8 (1.1)^{ax}
1997[1]	12.0 (1.2)^{ax}	6.7 (0.7)^{by}	12.3 (1.0)^{ax}	11.4 (1.3)^{ax}
% Change 1996 to 1997	+22	-36	+6	+16
1998[1]	10.8 (1.0)^{abx}	2.5 (0.6)^{cy}	11.6 (1.0)^{ax}	11.1 (1.2)^{ax}
% Change 1996 to 1998	+10	-75	0	+13
1999[1]	11.1 (0.8)^{abx}	0.8 (0.2)^{cy}	6.5 (0.8)^{bz}	10.5 (1.0)^{ax}
% Change 1996 to 1999	+13	-92	-44	+7
2000[1]	7.6 (0.8)^{bx}	0.6 (0.3)^{cy}	2.1 (0.2)^{cy}	11.8 (0.8)^{ax}
% Change 1996 to 2000	-22	-94	-82	+20
2001[1]	7.0 (0.7)^{bx}	0.5 (0.2)^{cy}	1.2 (0.2)^{cy}	7.3 (0.8)^{ax}
% Change 1996 to 2001	-29	-95	-90	-26

[1] Years with the same letter within each treatment are not significantly different ($P>0.05$) (a, b, and c).
[2] Treatments with the same letter are not significantly different ($P>0.05$) (x, y, and z).

Table 2. Forb and shrub species density/2.7 ft^2 quadrat on the cattle only non-infested (CON), cattle only leafy spurge infested (COS), sheep only non-infested (SON), sheep only leafy spurge infested (SOS), cattle and sheep non-infested (CSN), cattle and sheep leafy spurge infested (CSS), control non-infested (NUN), and control leafy spurge infested (NUS) treatments for 1996 through 2001. (SE in parentheses.)

	1996[1]	1997[1]	1998[1]	1999[1]	2000[1]	2001[1]
	Density/2.7 ft^2 quadrat					
CON[2]	6.7 (1.0)^{abxz}	----	4.1 (0.1)^{ax}	7.8 (1.1)^{bcxz}	9.5 (1.5)^{cz}	5.9 (0.7)^{abxz}
COS[2]	1.8 (0.4)^{ay}	1.5 (0.4)^{ax}	1.3 (0.3)^{ax}	1.0 (0.3)^{ay}	1.1 (0.3)^{ay}	2.3 (0.5)^{ay}
SON[2]	5.8 (1.1)^{abxz}	----	2.1 (0.5)^{bcx}	7.0 (1.5)^{ax}	6.0 (0.9)^{abx}	3.0 (0.4)^{bcx}
SOS[2]	1.1 (0.3)^{ay}	0.5 (0.2)^{ax}	0.8 (0.2)^{ax}	2.2 (0.5)^{ay}	1.7 (0.2)^{ay}	1.3 (0.2)^{axy}
CSN[2]	4.5 (0.4)^{ax}	----	2.3 (0.4)^{ax}	3.0 (0.4)^{ay}	3.5 (0.5)^{ay}	2.6 (0.3)^{ax}
CSS[2]	0.9 (0.2)^{ay}	0.3 (0.1)^{ax}	0.8 (0.3)^{ax}	1.4 (0.4)^{ay}	0.9 (0.4)^{ay}	0.8 (0.2)^{axy}
NUN[2]	7.8 (0.9)^{az}	----	6.9 (0.8)^{az}	5.8 (0.7)^{az}	6.1 (0.7)^{ax}	6.9 (0.8)^{az}
NUS[2]	1.1 (0.4)^{ay}	0.9 (0.3)^{ax}	1.0 (0.3)^{ax}	1.9 (0.5)^{ay}	1.6 (0.4)^{ay}	2.4 (0.7)^{ax}

[1] Years with the same letter within each treatment are not significantly different (P>0.05) (a and b).
[2] Treatments with the same letter are not significantly different (P>0.05) (x, y, and z).

Table 3. Shannon Weiner diversity index on the cattle only non-infested (CON), cattle only leafy spurge infested (COS), sheep only non-infested (SON), sheep only leafy spurge infested (SOS), cattle and sheep non-infested (CSN), cattle and sheep leafy spurge infested (CSS), non-use control non-infested (NUN), and non-use control leafy spurge infested (NUS) treatments for 1996, 1997, 1998, 1999, and 2000. (SE in parentheses.)

	1996[1]	1997[1]	1998[1]	1999[1]	2000[1]	2001[1]
	Species Diversity Index					
CON[2]	2.73 (0.17)^{ax}	---	2.60 (0.10)^{ax}	2.60 (0.05)^{ax}	2.65 (0.14)^{ax}	2.71 (0.11)^{ax}
COS[2]	2.30 (0.07)^{ay}	2.23 (0.26)^{ay}	2.12 (0.13)^{ay}	2.11 (0.19)^{ay}	2.26 (0.13)^{ay}	2.21 (0.08)^{ay}
SON[2]	2.62 (0.04)^{ax}	---	2.42 (0.25)^{ax}	2.58 (0.25)^{ax}	2.69 (0.19)^{ax}	2.55 (0.21)^{ax}
SOS[2]	2.31 (0.13)^{ay}	2.17 (0.21)^{ay}	2.24 (0.15)^{ay}	2.23 (0.18)^{ay}	2.37 (0.10)^{ay}	2.28 (0.11)^{ay}
CSN[2]	2.66 (0.17)^{ax}	---	2.46 (0.06)^{ax}	2.46 (0.08)^{ax}	2.63 (0.12)^{ax}	2.51 (0.09)^{ax}
CSS[2]	2.15 (0.12)^{ay}	1.91 (0.07)^{ay}	1.92 (0.21)^{ay}	2.19 (0.07)^{ay}	2.17 (0.04)^{ay}	2.23 (0.11)^{ay}
NUN[2]	2.57 (0.11)^{ax}		2.76 (0.12)^{ax}	2.67(0.15)^{ax}	2.76 (0.17)^{ax}	2.40 (0.35)^{ax}
NUS[2]	2.08 (0.04)^{ay}	1.92 (0.27)^{ay}	2.02 (0.29)^{ay}	1.90 (0.47)^{ay}	2.21 (0.28)^{ay}	2.24 (0.26)^{ay}

[1]Years with the same letter within each treatment are not significantly different (P>0.05)(a,b and c).
[2] Treatments with the same letter are not significantly different (P>0.05) (x, y, and z).

Table 4. Herbage production (lb/acre) on the non-use control treatment in 1996, 1997, 1998,1999, and 2000. (SE in parentheses.)

	1996[1]	1997[1]	1998[1]	1999[1]	2000[1]	2001[1]
	Lb/acre					
Grass & Grass-Like	1527 (146)^{a}	1317 (168)^{ab}	1060 (139)^{b}	1609 (202)^{a}	1652 (143)^{a}	2244 (93)^{c}
Forb	118 (43)^{ab}	87 (29)^{ab}	46 (23)^{a}	171 (59)^{b}	93 (53)^{ab}	92 (20)^{a}
Shrub	82 (76)^{a}	14 (13)^{a}	14 (14)^{a}	14 (12)^{a}	10 (8)^{a}	29 (14)^{a}
Leafy Spurge	407 (139)^{ab}	446 (77)^{ab}	350 (81)^{a}	410 (92)^{ab}	624 (173)^{b}	287 (45)^{a}

[1] Years with the same letter within each treatment are not significantly different (P>0.05) (a, b, and c)

Table 5. Livestock average daily gains (standard errors in parentheses) for individual livestock classes on the (CO) cattle only, (SO) sheep only, and (CS) cattle and sheep treatments for 1996, 1997, 1998, 1999, 2000, and 2001.

Treatment & Livestock Class[1]	1996[2]	1997[2]	1998[2]	1999[2]	2000[2]	2001[2]
	lb/day					
CO Steer	1.76 (0.07)^{ax}	1.61 (0.13)^{ax}	1.23 (0.06)^{ax}	1.80 (0.25)^{ax}	1.96 (0.24)^{ax}	1.86 (0.17)^{ax}
CS Steer	1.53 (0.32)^{ax}	1.12 (0.16)^{ax}	0.96 (0.13)^{ax}	1.44 (0.22)^{ax}	2.02 (0.10)^{ax}	1.63 (0.33)^{ax}
SO Ewe	0.16 (0.02)^{acx}	0.07 (0.02)^{bx}	0.04 (0.02)^{bx}	0.09 (0.02)^{abx}	0.20 (0.02)^{acx}	0.23 (0.02)^{cx}
CS Ewe	0.16 (0.02)^{abx}	0.09 (0.03)^{abx}	0.07 (0.02)^{bx}	0.18 (0.02)^{abx}	0.22 (0.03)^{ax}	0.20 (0.03)^{ax}

[1] Years with the same letter within each treatment are not significantly different (P>0.05) (a, b, and c).
[2] Treatments with the same letter within each livestock class are not significantly different (P>0.05) (x, y, and z).

CONCLUSIONS

Sheep grazing, either as a sole enterprise or mixed with cattle will provide and effective tool in controlling leafy spurge by reducing stem densities. When replacing cattle AUM's with sheep AUM's, leafy spurge stem density counts were reduced by 95% in five years of grazing. When grazing sheep and cattle together, leafy spurge was reduced by 90% in five years. There were no negative or positive effects on species diversity grazing sheep or cattle alone or together after five grazing seasons. Grass and grass-like disappearance was similar among all grazing treatments, showing replacing cattle with sheep would not affect grass and grass-like disappearance while reducing leafy spurge. There was no difference in livestock performance when grazing cattle and sheep separately or in combination, suggesting multi-species grazing had no negative or positive effects on livestock performance as it relates to weight gain in this study.

Literature Cited

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

Merrill, L.B., P.O. Reardon, and C.L. Leinweber. 1966. Cattle, sheep, goats... mix'em up for higher gains. Texas Agr. Prog. 12:13-14.

Prosser, C.W. 1995. Multi-species grazing of leafy spurge infested rangeland in North Dakota. M.S. Thesis. N.D. State Univ., Fargo. 80 pp.

Shiflets, T.N. 1994. Rangeland cover types of the United States. Soc. for Range Manage. Denver, CO. 152 pp.

SPSS. 2000. Statistical producers for Social Science. Marketing Dept. SPSS. Inc. Chicago, Ill.

Quimby, P.C. Jr., W.L. Bruckart, C.J. Deloach, L. Knutson, and M.H. Ralphs. 1991. Biological control of rangeland weeds. P 83-102 Chapter 9. In: Noxious Range Weeds. Westview Press, Boulder, San Francisco, & Oxford.

Whitman, Warren, C., D.W. Bolin, E.W. Klostermann, K.D. Ford, L. Moomaw, D.G. Hoag, and M.L.Buchanan. 1951. Caroten, protein, phosphorus in grasses of western North Dakota. Agr. Exp. Sta., N.D. Agr. College. N. D. Agr. Exp. Sta. Bull. No. 370.

EFFECTS OF MULTI-SPECIES GRAZING ON LEAFY SPURGE INFESTED RANGELAND USING TWICE-OVER ROTATION AND SEASON-LONG GRAZING TREATMENTS
(A Six-Year Summary)

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Acknowledgments: We would like to give special and well-deserved thanks to the Hettinger Research Extension Center staff (Don Stecher, Dennis Bentsen, Dave Pearson, Rick Olson, and Terri Lindquist). Also we would like to thank Dennis Whitted and Connie O'Brien for their help in collecting vegetative data.

Introduction

Herbicides continue to be the primary method to control and eradicate leafy spurge (*Euphorbia esula* L.) (Lym et al. 1995). However, it is not economically feasible to control large infestations (Bangsund et al. 1996). Most herbicides which provide effective control of leafy spurge are not labeled for use in environmentally sensitive areas. This noxious weed, which is extremely persistent and competitive, has contributed significantly to economic losses to the livestock industry (Leitch et al. 1994).

Use of grazing as a biological control for leafy spurge has become more acceptable in recent years. Goats have been shown to be an excellent tool to control and reduce leafy spurge infestations (Sedivec and Maine 1993, Hanson 1994, Prosser 1995, Sedivec et al. 1995). The use of sheep as a control method was proven as early as the late 1930s and early 1940s by Helgeson and Thompson (1939) and Helgeson and Longwell (1942). However, there have been many disagreements in the literature concerning utilization of leafy spurge by sheep (Landgraf et al. 1984) due to the aversive chemicals found in the latex of leafy spurge. Research by Lym and Kirby (1987) showed that cattle totally or partially avoid leafy spurge infested sites and intensify use on non-infested sites.

Multi-species grazing, the concurrent use of rangeland by more than one kind of animal has been advocated to maximize animal production (Merrill and Miller 1961). It is an important concept in rangeland management because of the presence of one or more classes of vegetation (Merrill et al. 1966). However, no published reports have documented the potential use of sheep and cattle in a multi-species grazing approach to improve graminoid species use, increase plant richness, and to control leafy spurge on leafy spurge infested rangeland.

The objectives of this study were to: 1) determine effects of multi-species grazing using twice-over rotation grazing system (TOR), season-long grazing treatments (SL), and non-use treatment

(NU) on leafy spurge control and 2) evaluate species diversity, herbage production, degree of disappearance of herbage and livestock performance on TOR and SL using a multi-species grazing program.

Study Area

The research was conducted on two separate tracts of land in Morton County. The first tract was Sections 31 and 32, T139N, R81W, in south central North Dakota, approximately two miles southwest of Mandan. This tract consisted of 603 acres of native rangeland owned by the North Dakota State Correctional Center. The second tract was on the north half of Section 9, T138N, R81W on 237 acres of native rangeland operated by the Northern Great Plains Research Laboratory, approximately three miles south of Mandan. Both tracts are found in the Missouri Slope Prairie Region and associated with the Heart River Watershed. Vegetation in this region is typical of northern mixed grass prairie (Barker and Whitman 1988) and classified as a wheatgrass-grama-needle grass (*Agropyron*, *Bouteloua*, *Stipa*) plant community (Shiflet 1994). Leafy spurge infestations were mapped before the study and estimated to cover 30 percent of each tract of rangeland.

The TOR consisted of four pastures grazed from 15 May to 1 October by one heard of cow/calf pairs and mature dry ewes. A total of 96 animal units (AU) of cattle (85 - 1200 lb. cows with calves) and 33 AU of sheep (200 - 135 lb. mature white-face ewes without lambs) or a total 532 animal unit month's (AUM's) grazed the TOR treatment in 1996 and 1997. Cattle AU were reduced to 85 AU of cattle (76 - 1200 lb. cows with calves) in 1998; however, sheep AU remained the same and a total 491 AUM's grazed the TOR in 1998. The overall stocking rate was 0.88 AUM's/acre in 1996 and 1997 and 0.82 AUM's/acre in 1998 on the TOR treatment. Stocking rates were decreased due to below average winter snow cover and rain fall in the spring 1998.

The SL treatment was grazed moderately light in 1996 due to lack of range evaluation data and unknown carrying capacities. Twenty-seven AU of cattle (35 - 700 lb. Yearling steers) and 8 AU of sheep (48 - 135 lb. mature white-face ewes without lambs) or a total 144 AUM's grazed the SL treatment in 1996. The overall stocking rate was 0.68 AUM's/acre in 1996 on the SL treatment. The SL treatment was grazed by yearling steers and mature ewes and stocked with 37 AU of cattle (49 - 705 lb. yearling steers) and 13 AU of sheep (78 - 135 lb. mature white-face ewes without lambs) or a total 207 AUM's grazed in 1997 and 1998. The overall stocking rate was 0.88 AUM's/acre in 1996, 1997, and 1998 on the SL treatment.

Sheep were placed on pasture approximately 15 May each year when leafy spurge was ready for grazing and cattle placed on pasture 1 June when native cool season grass species reach grazing readiness (3-4 leaf stage). Livestock species were removed from the treatments when 50 to 60 percent degree of graminoid disappearance was reached or 1 October. During all three years livestock grazed until 1 October.

Methods

Objective 1

Leafy spurge density was counted in six 32 ft by 16 ft enclosures. Three enclosures were systematically placed in each of the TOR and SL treatments. Each 32 ft by 16 ft enclosure was

subdivided in two 16 ft by 16 ft plots with one plot randomly assigned a grazed treatment (TOR or SL) and second plot an ungrazed treatment (NU). A 2.5 ft buffer was placed along the inside border of each grazed and ungrazed plot to prevent an edge effect. Each plot was further stratified into 12 inch^2 (0.1 m^2) quadrats and each quadrat assigned a number. Ten 1.08 ft^2 quadrats were randomly selected in each treatment for leafy spurge density counts. Leafy spurge densities were collected in the first week of June throughout the duration of the study.

Objective 2

Forage production and degree of disappearance for leafy spurge, grass and grass-like, shrubs, and other forbs were determined using a pair-plot clipping technique (Milner and Hughes 1968). Eight cages were dispersed in each of the four pastures of the TOR. Four of the cages were systematically placed in leafy spurge infested sites and four in non-infested sites. Twelve cages were systematically placed in the SL, six cages placed on leafy spurge infested sites and six cages on non-infested sites. Two plots were clipped from each cage using a 2.7 ft^2 (0.25 m^2) frames.

Livestock performance and production were determined for both cattle and sheep and expressed as average daily gain. Weights were taken when animals were allocated to and removed from each treatment.

Data Analysis

Treatment and year effects for leafy spurge stem density, species richness, forb and shrub density, herbage production, degree of use, and livestock performance were analyzed using a general linear model (GLM) (SPSS 1999). A mean separation was performed using Tukey's Honesty Significant Difference when significant ($P<0.05$) differences were found. The Shannon Wiener Index was used to calculate species diversity indices for both leafy spurge infested and non leafy spurge infested range sites. Treatment and year effect's of species diversity was analyzed using a non-parametric test (Krushal-Wallis Test) (SPSS 1999).

Results and Discussion

Leafy spurge stem density significantly decreased ($P\leq0.05$) on the SL after three grazing seasons, and took four grazing seasons to see a significant change ($P\leq0.05$) within the TOR. After five grazing seasons the SL had a reduction of 99% and the TOR had 28% reduction of leafy spurge stem densities. These results followed similar trends found by Lym et al. (1997) comparing multi-species grazing with cattle and angora goats. They reported season-long grazing reduced leafy spurge stem density faster than rotational grazing. Results of this study support Lym et al. (1997) in that season-long grazing using a multi-species approach will reduce leafy spurge stem density faster than rotational grazing.

Species diversity significantly changed ($P\leq0.05$) on both the TOR and SL native range sites (Table 2). Diversity significantly decreased ($P\leq0.05$) on the TOR native silty range site from 1996 to 2001. The SL native silty range site increased ($P\leq0.05$) in species diversity from 1996 to 2000. Species diversity did not change ($P>0.05$), however, on the leafy spurge infested range sites over five grazing seasons. Results also showed differences in species diversity between the TOR and SL treatments on native and leafy spurge infested range sites in years 1998 through 2001 (Table 2).

Results show significant changes ($P \leq 0.05$) in herbage production over six growing seasons on both the TOR and SL grazing treatments (Table 3 and 4). Grass and grass-like production on the SL leafy spurge infested and TOR non-infested sites have significantly increased ($P \leq 0.05$) from 1998 to 2001. Forb production also increased ($P \leq 0.05$) on the TOR non-infested sites from 1996 to 2000, however, there was significant decrease in forb production from the 2000 to 2001 growing seasons (Table 3). Leafy spurge production has decreased on both the TOR and SL. There were significant changes ($P \leq 0.05$) in leafy spurge production on the TOR, however, result show that there were no significant decreases ($P > 0.05$) on the SL (Table 3 and 4). Production on the SL leafy spurge infested sites was zero (Table 4)

Cow average daily gain (ADG) decreased ($P < 0.05$) from 1996 to 1998 and increased ($P \leq 0.05$) from 1998 to 2001. Cow ADG was significantly higher ($P \leq 0.05$) in 2000 than the other five grazing seasons. Calf ADG results showed a significant increase ($P \leq 0.05$) ADG from 1996 to 1999 and from 1999 to 2000, however, calf ADG significantly lower ($P \leq 0.05$) in 2001 than 2000 (Table 5). Steer ADG results on the SL treatment also showed a significant decrease ($P \leq 0.05$) in 1998 and then an increase ($P \leq 0.05$) in 1999 (Table 5). Average daily gains decreased ($P \leq 0.05$) from 1999 to 2000 and from 2000 to 2001.

Ewe average daily gain (ADG) on the TOR significantly decreased ($P \leq 0.05$) from 1996 to 1999, and increased ($P \leq 0.05$) from 1999 to 2000, however, ewe ADG in 2001 was lower ($P \leq 0.05$) than 2000 (Table 5). Ewe ADG results on the SL also showed up and down ADG throughout six growing seasons. Ewe ADG increased ($P \leq 0.05$) from 1996 to 1997 and then significantly decreased ($P \leq 0.05$) from 1997 to 1998 and 1998 to 1999, however, ADG significantly increased ($P \leq 0.05$) from 2000 to 2001 (Table 5). Ewe ADG results also showed that the TOR had higher ($P \leq 0.05$) ewe ADG than the SL in years 1996, 1999, and 2000, and that the SL had a higher ($P \leq 0.05$) ewe ADG than the TOR in 2001 (Table 5). Average daily gains for ewes were similar ($P > 0.05$) among the TOR and SL in 1997 and 1998.

Table 1. Leafy spurge stem densities on the season-long (SL), twice-over rotation (TOR) grazing treatment, and ungrazed treatments (NU) for 1996 through 2001. (SE in parentheses.)

	Season-long	Twice-Over Rotation
	---------- # / 1.08 ft^2----------	
1996	14.4 (1.9)[a]	13.2 (1.5)[a]
1997	12.5 (1.0)[a]	15.9 (1.4)[a]
% change 1996 to 1997	**-13.2**	**+20.5**
1998	11.5 (1.5)[a]	12.8 (1.1)[a]
% change 1996 to 1998	**-20.1**	**-3.0**
1999	5.7 (0.6)[b]	13.4 (1.4)[ac]
% change 1996 to 1999	**-60.4**	**+1.0**
2000	1.1 (0.3)[b]	9.0 (1.3)[c]
% change 1996 to 2000	**-92.3**	**-31.8**
2001	0.1 (0.05)[b]	9.5 (0.7)[c]
% change 1996 to 2001	**-99.3**	**-28.0**

[1] **Years and treatments with the same letter within treatments are not significantly different (P>0.05)**

Table 2. Shannon Weiner diversity index on the twice-over rotation and season-long treatments for 1996 through 2001 (SE in parentheses).

Treatments & Sites	1996	1997	1998	1999	2000	2001
	----------Species Diversity Index----------					
Twice-Over						
Native Shallow	2.55 (0.13)^{ax}	---	2.36 (0.22)^{ax}	2.36 (0.08)^{ax}	2.04 (0.10)^{ax}	2.23 (0.22)^{ax}
Leafy Spurge Shallow	2.25 (0.18)^{ax}	2.09 (0.15)^{ay}	2.19 (0.17)^{ax}	2.39 (0.26)^{ax}	2.15 (0.40)^{ax}	2.26 (0.25)^{ax}
Native Silty	2.62 (0.09)^{ax}	---	2.44 (0.08)^{ax}	2.34 (0.21)^{abx}	2.07 (0.09)^{bx}	2.08 (0.10)^{bx}
Leafy Spurge Silty	2.19 (0.06)^{ay}	2.09 (0.04)^{ay}	2.39 (0.24)^{ax}	2.31 (0.29)^{ax}	2.28 (0.17)^{ax}	2.35 (0.04)^{ax}
Season-long						
Native Shallow	2.98 (0.11)^{abz}	---	3.01 (0.09)^{bz}	2.65 (0.25)^{ax}	3.06 (0.10)^{bz}	2.84 (0.17)^{abz}
Leafy Spurge Shallow	2.12 (0.02)^{ay}	1.99 (0.17)^{ay}	1.94 (0.11)^{ay}	2.14 (0.24)^{ay}	2.04 (0.39)^{ay}	2.38 (0.09)^{ay}
Native Silty	2.69 (0.13)^{abx}	---	2.52 (0.09)^{ax}	2.69 (0.09)^{abx}	2.83 (0.08)^{bz}	2.78 (0.08)^{bz}
Leafy Spurge Silty	2.15 (0.01)^{ay}	2.04 (0.13)^{ay}	2.22 (0.09)^{ax}	2.15 (0.06)^{ay}	2.00 (0.02)^{ay}	2.07 (0.25)^{ay}

[1] Years with the same letter within each treatment are not significantly different (P>0.05) (a, b, and c).
[2] Treatments with the same letter within each year are not significantly different (P>0.05) (x, y, and z).

Table 3. Herbage production and degree of disappearance (%) on the twice-over rotation (TOR) treatment from 1996 through 2001.

	1996		1997		1998		1999		2000		2001	
	lb/acre	% Use	lb/acre	% Use	lb/acre	% Use	lb/acre	% Use	lb/acre	% Use	lb/acre	% Use
Non-infested sites												
Grass and Grass-Like	2468±177	34	1883±156	37	1380±89	24	2165±386	18	2216±237	38	2969±201	46
Forb	143±37	0	121±37	51	105±19	36	403±123	69	863±230	42	117±74	70
Shrub	6±6	100	0±0	0	0±0	0	2±2	0	0±0	0	0±0	0
Total	**2619±178**	**32**	**2004±148**	**38**	**1484±91**	**25**	**2569±389**	**26**	**3079±328**	**45**	**3087±171**	**17**
	1996		1997		1998		1999		2000		2001	
	lb/acre	% Use	lb/acre	% Use	lb/acre	% Use	lb/acre	% Use	lb/acre	% Use	lb/acre	% Use
Leafy Spurge infested sites												
Grass and Grass-Like	1268±113	2	1280±130	20	1291±154	27	1802±270	25	1502±266	11	2090±190	22
Forb	1±1	0	35±28	89	36±12	83	147±55	22	1009±316	1	11±9	100
Shrub	0±0	0	0±0	0	0±0	0	0±0	0	0±0	0	0±0	0
Leafy Spurge	1144±172	41	955±117	61	776±100	61	1325±203	73	1603±337	41	731±132	69
Total	**2413±173**	**20**	**2270±138**	**38**	**2103±177**	**40**	**3274±391**	**42**	**4114±481**	**46**	**2832±236**	**35**

Table 4. Herbage production and degree of disappearance (%) on the season-long (SL) treatment from 1996 through 2001.

	1996		1997		1998		1999		2000		2001	
	lb/acre	% Use	lb/acre	% Use	lb/acre	% Use	lb/acre	% Use	lb/acre	% Use	lb/acre	% Use
Non-infested sites												
Grass and Grass-Like	2784±306	21	2043±333	32	1803±143	37	2323±352	43	1896±127	34	2672±371	31
Forb	384±75	0	162±73	71	119±41	32	280±72	61	355±138	63	52±35	65
Shrub	8±8	55	33±18	100	5±5	20	0±0	0	0±0	0	0±0	0
Total	**3166±270**	**15**	**2237±308**	**36**	**1927±167**	**37**	**2603±385**	**45**	**2250±247**	**39**	**2724±398**	**31**
	1996		1997		1998		1999		2000		2001	
	lb/acre	% Use	lb/acre	% Use	lb/acre	% Use	lb/acre	% Use	Lb/acre	% Use	lb/acre	% Use
Leafy Spurge infested sites												
Grass and Grass-Like	1713±154	1	1239±169	33	817±124	40	1701±300	39	2832±306	47	3150±376	39
Forb	6±6	0	2±2	0	0±0	0	137±88	85	0±0	0	0±0	0
Shrub	0±0	0	8±8	100	0±0	0	0±0	0	0±0	0	0±0	0
Leafy Spurge	857±165	47	823±89	47	480±109	46	471±225	89	29±19	100	0±0	0
Total	**2576±125**	**16**	**2703±190**	**35**	**1297±151**	**42**	**2308±162**	**52**	**2861±299**	**50**	**3150±376**	**39**

Table 5. Livestock average daily gains for individual classes of livestock on treatments: twice-over rotation (TOR) and season-long (SL) for 1996 through 2001. (SE in parentheses.)

Treatment & Livestock Class[1]	1996[2]	1997[2]	1998[2]	1999[2]	2000[2]	2001[2]
	----------lb/day ----------					
TOR						
Cow	0.78 (0.05)^{a}	1.00 (0.05)^{b}	0.01 (0.04)^{c}	0.67 (0.05)^{a}	1.39 (0.05)^{d}	0.85 (0.05)^{ab}
Calf	2.33 (0.03)^{a}	2.32 (0.03)^{a}	2.42 (0.03)^{a}	2.64 (0.03)^{b}	2.86 (0.02)^{c}	2.55 (0.03)^{b}
Ewe	0.32 (0.01)^{ax}	0.25 (0.01)^{bx}	0.26 (0.01)^{bx}	0.24 (0.01)^{bx}	0.30 (0.004)^{ax}	0.20 (0.003)^{cx}
SL						
Steer	1.99 (0.04)^{ad}	1.84 (0.03)^{ab}	1.54 (0.04)^{c}	2.09 (0.04)^{d}	1.91 (0.22)^{a}	1.78 (0.03)^{b}
Ewe	0.23 (0.03)^{acy}	0.28 (0.03)^{cx}	0.22 (0.01)^{ax}	0.17 (0.01)^{by}	0.18 (0.006)^{by}	0.32 (0.01)^{dy}

[1] Years with the same letter within each treatment are not significantly different (P>0.05) (a, b, c, and d).
[2] Sheep (ewe) treatments with the same letter within each year are not significantly different (P>0.05) (x and y).

Conclusion

Multi-species grazing with cattle and sheep in a season-long (SL) grazing treatment will reduce leafy spurge quicker than a twice-over rotation (TOR) grazing treatment. The trend of this study, however, would show that in time the TOR would provide similar control than the SL in a long term management plan. The continuation of this project will allow use to detect which treatment will increase plant species diversity on leafy spurge infested sites. At this time it is too soon to make any conclusion on species diversity. Livestock performance results showed that the TOR has provided greater average daily gains than the TOR for the ewes, however, this may be related to the amount of leafy spurge remaining in the TOR. Leafy spurge stem counts on the TOR are still much higher than the SL, which would suggest that the ewes on the TOR are receiving a higher quality diet than the SL throughout the growing season with the presence of leafy spurge, however, this doesn't hold true for the 2001 grazing season where ewe ADG was higher on the SL treatment. Livestock results have also shown that the TOR has increased calf average daily gains over five grazing seasons.

Literature Cited

Bangsund, D.A., J.A. Leitch, and F.L. Leistritz. 1996. Economic analysis of herbicide control of leafy spurge in rangeland. N.D. State Univ. Agr. Exp. Sta., Agr. Econ. Rep. No. 342-S. Fargo. 11 p.

Bangsund, D.A., D.J. Nudell, R.S. Sell, and F.L. Leistritz. 2000. Economic analysis of Controlling leafy spurge with sheep. N.D. Agr. Exp. Stat. N.D. Univ. Agr. Econ. Rep. No. 431-S. Fargo.

Barker, W.T. and W.C. Whitman. 1988. Vegetation of the northern Great Plains. Rangelands. 10:266-272.

Hanson, T.P. 1994. Leafy spurge control using Angora goats. M.S. Thesis. N.D. State Univ. Fargo. 111 p.

Helgeson, E.A. and E.J. Thompson. 1939. Control of leafy spurge by sheep. N.D. Agr. Exp. Sta. Bimonthly Bull. 2(1):5-9.

Helgeson, E.A. and E.J. Longwell. 1942. Control of leafy spurge by sheep. N.D. Agr. Exp. Sta. Bimonthly Bull. 4(5):10-12.

Landgraf, B., P.K. Fay, and K.M. Havstad. 1984. Utilization of leafy spurge by sheep. Weed Sci. 32(3):348-352.

Leitch, J.A., F.L. Leistritz, and D.A. Bangsund. 1994. Economic effect of leafy spurge in the upper Great Plains: methods, models, and results. Agr. Econ. Rep. No. 316. N.D. State Univ. Agr. Econ. Dept. Fargo.

Lym, R.G. and C.G. Messersmith. 1987. Carbohydrates in leafy spurge roots as influenced by environment. J. Range Manage. 40:139-144.

Lym, R.G., C.G. Messersmith, and R. Zollinger. 1995. Leafy spurge identification and control. N.D. State Univ. Ext. Serv. Circ. W-765 (revised). Fargo. 4 p.

Lym, R.G., K.K. Sedivec, and D.R. Kirby. 1997. Leafy spurge control with angora goats and herbicides. J. Range Manage. 50:123-128.

Merrill, L.B. and Miller. 1961. Economic analysis of year long grazing rate studies on substation No.14 near Sonora. Texas Agr. Prog. 12:13-14.

Merrill, L.B., P.O. Reardon, and C.L. Leinweber. 1966. Cattle, sheep, goats... mix'em up for higher gains. Texas Agr. Prog. 12:13-14.

Milner, C. and R.E. Hughes. 1968. Methods of the measurement of primary production of grassland. Blackwell Sci. Publ., Oxford, England.

Prosser, C.W. 1995. Multi-species grazing of leafy spurge infested rangeland in North Dakota. M.S. Thesis. N.D. State Univ. Fargo. 80 p.

Sedivec, K.K. and R.T. Maine. 1993. Angora goat grazing as a biological control for leafy spurge infested rangeland. In: Proc. 1993 Leafy Spurge Symp. Cranby, Colo.

Sedivec, K.K., W.T. Barker, and C.W. Prosser. 1995. Intensive grazing of Angora goats on leafy spurge infested rangeland. p. 34-36. In: Proc. Leafy Spurge Symp. Fargo, N.D.

Shiflets, T.N. 1994. Rangeland cover types of the United States. Soc. for Range Manage., Denver, CO. 154 pp.

SPSS. 1999. Statistical producers for Social Science. Marketing Dept. SPSS. Inc. Chicago, Ill.

CATTLE AND SHEEP GRAZING LEAFY SPURGE (*Euphorbia esula* L.) INFESTED RANGELAND

A DEMONSTRATION GRAZING TRIAL ON THE FORT BERTHOLD INDIAN RESERVATION

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Acknowledgments: *We would like to give special and well-deserved thanks to Ethan Hall for supplying the cow/calf pairs and the tract of land. We would also like to thank Luke W. Samuel, Mitch D. Faulkner, and Joseph E. Hochhalter for their assistance in collecting vegetative data for this project.*

Introduction

Weedy forbs and brush cause more losses on the United States 252 million hectares of rangeland than all other pests combined (Quimby et al. 1991), in which herbicides continue to be the main method of controlling these plant species. Herbicides are effective in controlling noxious weeds and other troublesome plant species, however, due to environmental constraints and costs of herbicides many land managers are forced to use alternative methods. Leafy spurge (*Euphorbia esula* L.) is just one of many plant species that cause economic burden to land managers and the rural communities.

The use of sheep as a biocontrol agent in the control of leafy spurge is not a new concept. In the late 30's and early 40's Christensen et al. (1938), Helgeson and Thompson (1939) and Helgeson and Longwell (1942) indicated that sheep consumed leafy spurge and should be integrated into management strategies in controlling leafy spurge, however, there was limited promotion. Herbicides continue to be the primary method for control of leafy spurge (Lym et al. 1995). Many areas infested with leafy spurge, however, are in environmentally sensitive areas and most herbicides for controlling leafy spurge are not labeled for application in these sensitive areas. Which have lead many land managers to choose an alternative control agent such as Angora goats and sheep. Research conducted in the 1980's and 1990's has shown that sheep or goats will reduce leafy spurge stem densities and increase grass and grass-like disappearance, and there are significant benefits in using multi-species grazing to manage leafy spurge infested rangelands (Prosser 1995).

Multi-species grazing is an important idea in rangeland management because rangelands usually consist of one or more classes of vegetation (Merrill et al. 1966). By using more than one livestock species on a given rangeland containing various vegetative communities provides the potential of increasing red meat production, species diversity, vegetative production, and revenue for a given ranching operation, with proper management plans. Although multi-species grazing provides the above benefits, the introduction of leafy spurge and its consistency of infesting grasslands in the mid-west exploit the importance of using a multi-species grazing approach.

The objectives of this demonstration trial were to demonstrate that sheep are effective in controlling leafy spurge and to demonstrate the benefits of using a multi-species grazing approach.

Study Area

A Demonstration grazing trial was conducted on the SE ¼ of Section 8, SW ¼ of Section 9, and NW ¼ of Section 16, T147N, R93W of Mckenzie County, approximately 17 miles southwest of Mandaree, North Dakota. Vegetation in this region is typical of the northern mixed grass prairie (Barker and Whitman 1988) and classified as a wheatgrass-grama-needle grass (*Agropyron, Bouteloua, Stipa*) plant community (Shiflet 1994). Approximately 15 percent of this tract of land was infested by leafy spurge before the on set of the demonstration grazing trial.

The demonstration grazing trial consisted of a 307.2 acre tract of land subdivided into a three pasture twice-over rotational grazing treatment (TOR) and a control. The TOR was grazed approximately from the 3rd week of June up until the 15th of September by one herd of cow/calf pairs and mature dry white-face ewes. A total of 38 animal units of cattle (33 - 1200 lb. cows with calves) and 10 animal units of sheep (60-135 lb. mature white-face ewes without lambs) or a total 120 AUMs grazing on the treatment in 2000 and 2001.

Methods

Leafy spurge stem densities were obtained using a 2.7 ft^2 quadrat along four 55 yard systematically placed line transects. Transects were selected based on leafy spurge location and data was collected every 2 ½ yards (20 readings/transect). Leafy spurge stem counts were also collect within a 32 ft x 16 ft enclosure (control). Twenty readings were collected within this enclosure by subdividing the enclosure into 2.7 ft^2 plots and randomly selecting 20 plots. Treatment and year effects for leafy spurge stem density were analyzed using a general linear model (GLM) (SPSS 1999).

Herbage production and degree of disappearance for leafy spurge, graminoid, shrubs, and other forbs were determined using a pair-plot technique (Milner and Hughes 1968). Ten cages were systematically dispersed in two of the three pastures of the TOR. Two plots were clipped from within each cage and two out of the cage using a 2.7 ft^2 quadrat. Samples were oven dried at 140 °F for 48 hours and weighed. Treatment and year effects for herbage production were analyzed using a general linear model (GLM) (SPSS 1999).

Results

No significant ($P>0.05$) changes occurred in leafy spurge stem densities on the TOR or the Control from 2000 to 2001. Leafy spurge stem densities were also similar ($P>0.05$) between the control and the TOR in 2000 and 2001 (Table 1).

Results showed that there were no significant ($P>0.05$) change in graminoid, forb, and shrub herbage production after one year of treatment on the TOR. Leafy Spurge production, however, was significantly ($P<0.05$) lower in the 2001 growing season than the 2000 growing season (Table 2). Table 2 also shows that there were increases in leafy spurge and shrub degree of disappearance in the 2001 grazing season on the TOR.

Table 1. Leafy spurge stem densities on the Fort Berthold Indian Reservation multi-species grazing demonstration study, in 2000 and 2001. (Standard errors are with in parentheses.)

Treatment	2000[1]	2001[1]
	# of Stems/2.7 ft^2 Frame	
Control[2]	6.25 (1.13)^{ax}	8.15 (1.17)^{ax}
Twice-over Rotation[2]	5.87 (0.93)^{ax}	5.93 (0.52)^{ax}

[1]Years with the same letter within each treatment are not significantly different (P>0.05) (a, b, and c).
[2] Treatments with the same letter are not significantly different (P>0.05) (x, y, and z).

Table 2. Herbage production and degree of disappearance on graminoid, forb, shrub, and Leafy spurge in 2000 and 2001.

	2000		2001	
	lb/acre	%disappearance	lb/acre	%disappearance
Graminoids	1461.1^a	64.0%	1545.5^a	47.6%
Forbs	174.8^a	79.3%	78.2^a	20.2%
Shrubs	68^a	55.0%	119^a	100.0%
Leafy Spurge	469.2^a	68.6%	269^b	84.2%

Conclusion

Results showed that there were no significant changes in leafy spurge stem densities, however, it did show that there was a significant decrease in leafy spurge herbage production from 2000 to 2001. Degree of disappearance of leafy spurge also increased in the 2001 grazing season. Based on these results multi-species grazing in a twice-over rotational grazing system decreased the production of leafy spurge, however, it also indicates under a multi-species grazing approach in twice-over rotation grazing system it takes more than two years of grazing to see significant changes in leafy spurge stem density. These findings complement the findings of the Hettinger Research Extension Centers and North Dakota State University, Animal and Range Sciences Department on the use of cattle and sheep in a multi-species approach to control leafy spurge.

Literature Cited

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

Christensen, F.W., T.H. Hopper, E.A. Helgeson, and E.J. Thompson. 1938. Leafy spurge as a feed for sheep. Amer. Soc. Anim. Prod. 31:311-316.

Helgeson, E.A. and E.J. Thompson. 1939. Control of leafy spurge by sheep. N.D. Agr. Exp. Sta. Bimonthly Bull. 2(1):5-9.

Helgeson, E.A. and E.J. Longwell. 1942. Control of leafy spurge by sheep. N.D. Agr. Exp. Sta. Bimonthly Bull. 4(5):10-12.

Merrill, L.B., P.O. Reardon, and C.L. Leinweber. 1966. Cattle, sheep, goats... mix'em up for higher gains. Texas Agr. Prog. 12:13-14.

Milner, C. and R.E. Hughes. 1968. Methods of the measurement of primary production of grassland. Blackwell Sci. Publ., Oxford, England.

Shiflets, T.N. 1994. Rangeland cover types of the United States. Soc. for Range Manage. Denver, CO. 152 pp.

SPSS. 1999. Statistical producers for Social Science. Marketing Dept. SPSS. Inc. Chicago, Ill.

Quimby, P.C. Jr., W.L. Bruckart, C.J. Deloach, L. Knutson, and M.H. Ralphs. 1991. Biological control of rangeland weeds. P 83-102 Chapter 9. In: Noxious Range Weeds. Westview Press, Boulder, San Francisco, & Oxford.

Effects of Winter Grazing on Herbage Production

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Introduction

Winter or dormant-season grazing is practiced by many western Dakota livestock producers in an effort to lower feed costs. Although adequate information exists regarding nutritional management of winter grazing cattle, little is known about the ecological effects of these practices. Furthermore, research emphasizing inferences for specific winter-grazing management is lacking. The objectives of this study are to determine impacts of winter grazing on herbage production, growth rate of dominate grass species (short-term), and changes in plant-species composition (long term) under various levels of winter and summer utilization.

Study Area and Design

Research was initiated on native rangeland in the western Dakotas in 2000. Study areas were selected in Adams County, North Dakota approximately 5 miles southwest of Hettinger and Perkins County, South Dakota approximately 15 miles south of Lodgepole. Vegetation was described as the wheatgrass-grama-needle grass association with a forage base of predominately native prairie comprised of blue grama (*Bouteloua gracilis*), western wheatgrass (*Agropyron smithii*), needle-and-thread (*Stipa comata*), and thread-leaf sedge (*Carex filifolia*) (Barker and Whitman 1989, Shiflets 1994).

Each study area was blocked into 4 pastures with the treatments 50% summer season-long use (control), 25% summer use for 2 weeks in June and 50% winter utilization (flash grazing use), 30% winter utilization, and 50% winter utilization randomly assigned. Stocking rates for season-long pastures were determined using the Soil Conservation Service Technical Guide (1984) using the Missouri slope vegetation zone. Winter pasture stocking rates were determined by collecting standing plant biomass prior to turnout from 10 randomly placed 0.25m^2 quadrats on both silty and shallow range sites (n=20) from each replicate to determine total available forage for each pasture. Final stocking rates for each treatment were computed by calculating a 25% grazing use efficiency with 50% disappearance (Laycock et al. 1972, Pearson 1975) and a dry-matter intake for a 1,200 lb dry brood cow using the Beef National Research Council (1996) guidelines. Winter grazing cattle were allowed ad libitum use of salts and minerals and were supplemented with 3 lbs/head/day of 30% crude protein all-natural cake. Cattle grazed as snow cover allowed for 53 days beginning November 15th.

Seasonal forage availability was determined by clipping 10 randomly placed 0.25m^2 frames from non-grazed shallow and silty range sites (n=20) on each study area. Samples were collected from June to November and as conditioned allowed in February and April.

Twenty 2.5 ft^2 sites within each treatment found to be indicative of the predominate forage base were randomly selected and protected from grazing in 2001, following one season of use. A 0.25 m^2 quadrat was clipped from each site in mid July to determine peak herbage production on each treatment (n=20).

Treatment effects were analyzed using a General Linear Model (GLM) and univariate analysis of variance (SPSS 2000). When differences were found, a means seperation was conducted using a Tukey's Honesty Significant Difference Test (Steel and Torrie 1980, SPSS 2000). Locations were tested for differences in herbage production potential using a GLM for block effect. When block effects were not detected, locations were combined and tested for overall treatment effect.
Data was analyzed to determine differences at the 0.05 percentile (P(0.05)). Adjusted R^2 and standard error values were calculated for herbage growth patterns and derived using a best fit curve (quadratic) estimation analysis (SPSS 2000).

Results

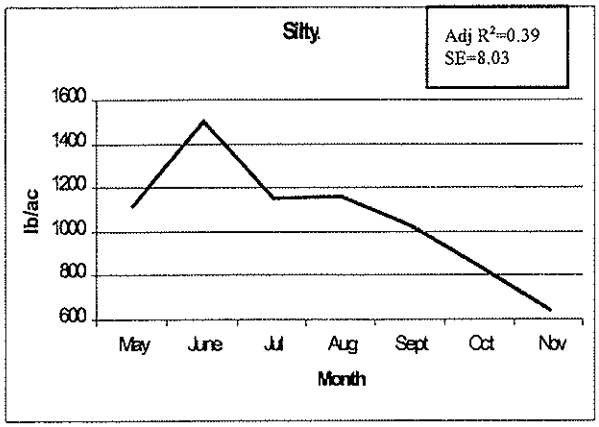
No differences in herbage production were found between locations($P=0.296$, $F=1.097$). Following one grazing season, peak primary production on the winter treatments was not lower than the season-long (control) pasture ($P>0.05$). Furthermore, herbage production was higher ($P\leq0.01$) on the flash gazing treatment than on the season-long and winter-only treatments (Table 1).

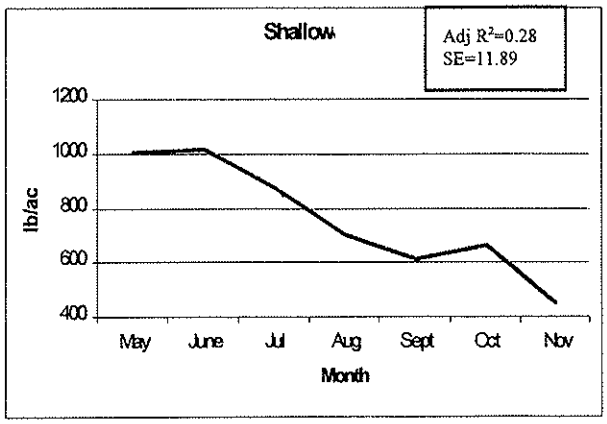
Table 1. Comparison of herbage production between grazing treatments in western North and South Dakota in 2001.

Treatment	lb/ac	% Difference
Season-long (Summer use)	1252[a]	0
Flash (25% use in June and 50% use in winter)	1579[b]	+26
30% Winter use	1162[a]	-7
50% Winter use	1326[a]	+6

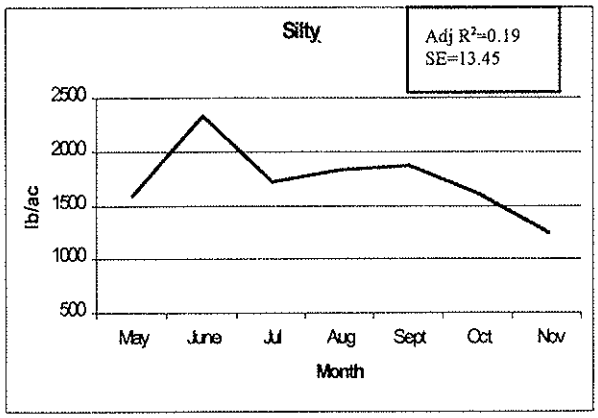
Treatments with same letter are not significantly different ($P>0.05$).

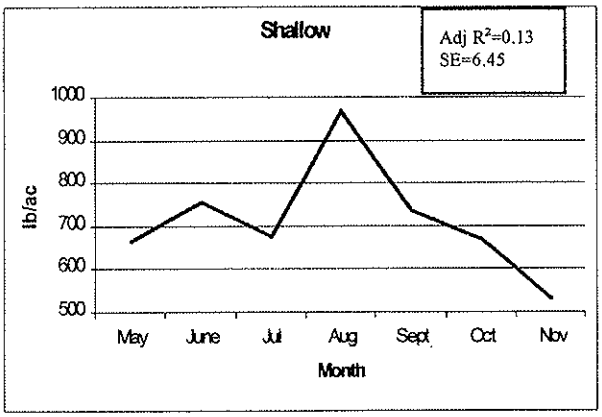
Figures 1a through 2b display mean lb/ac and corresponding regression analysis of available forage from late May through mid November on silty and shallow range sites for both study areas. Available forage began declining on the North Dakota sites in June and August, ranging from 1,500 lb/ac in June to 645 in November on the silty range site and from 1,010 lb/ac in June to 451 in November on the shallow range site. The South Dakota sites were reduced from 2,340 lb/ac in June to 1,234 in November on the silty range site and from 970 lb/ac in August to 532 in November on the shallow range site.





Figures 1a and b. Standing biomass (potential forage) on silty and shallow range sites in North Dakota.





Figures 2a and b. Standing biomass (potential forage) on silty and shallow range sites in South Dakota.

Summary

Peak herbage production as measured by above-ground standing biomass was not negatively affected by winter grazing at both 30 and 50% utilization after one grazing season. Brief early-summer grazing (June) in conjunction with 50% winter grazing appeared to have a beneficial effect on herbage production versus winter grazing only, thus providing a beneficial alternative to conventional forage stockpiling by deferring pastures until the beginning of the winter grazing season. Furthermore, this method minimizes forage lost from senescence from early summer to the beginning of the winter grazing season.

Literature Cited

Barker, W.T. and W.C. Whitman. 1989. Vegetation of the Northern Great Plains. NDSU Research Rpt. No. 111. Fargo.

Laycock, W.A., H. Buchanan, and W.C. Krueger. 1972. Three methods of determining diet, utilization, and trampling damage on sheep ranges. J. Range Manage. 25:352-356.

Nutrient Requirements for Beef Cattle. 1996. Nutrient requirements for beef cattle (7th ed.) Nat. Acad. Of Sciences–NRC, Washington, D.C.

Pearson, H.A. 1975. Herbage disappearance and grazing capacity determinations of southern pine bluestem ranges. J. Range Manage. 28:71-73.

SPSS. 2000. Statistical Producers for Social Science. Marketing Dept. SPSS. Inc. Chicago, Ill.

Shiflets, T.N. 1994. Rangeland cover types of the United States. Soc. For Range Manage., Denver, CO. 154 pp.

Steel and Torrie. 1980. Principles and procedures of statistics. (2nd Edition), McGraw-Hill, New York Company. New York, NY.

USDA Soil Conservation Service. 1984. Technician's guide to range sites and condition classes in North Dakota. USDA Soil Conservation Service, North Dakota Office, Bismarck.

ND 1709 Objective 2

GENETIC AND ENVIRONMENTAL STRATEGIES TO IMPROVE THE EFFICIENCY OF LEAN TISSUE ACCRETION IN LAMBS: 2001 UPDATE: LEAN LAMB PRODUCTION

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The primary focus of 2001 was to continue to accumulate data on offspring of the seven sires identified through Bioelectical Impedance Analysis (BIA) in 1999. These rams were selected based on estimated lean tissue accretion per day of age and the ratio of lean per day of age to estimated fat per day of age. The procedure by which the rams were selected has been described in previous Western Dakota Sheep Day Reports. Based on BIA data, rams were assigned as "lean" or "fat" sires.

A group of Western White-faced ewes was acquired as mates for the selected rams. The flock was managed as a typical commercial ewe flock and the resulting offspring fed to acceptable slaughter weights under typical feedlot conditions. Lambs were shipped to Iowa Lamb Company at Hawarden, Iowa, for slaughter and carcass evaluation. End-point weight was a minimum of 125 pounds (at Hettinger) as Iowa Lamb does not allow "breaking" of carcasses which weigh less than 56 pounds. Because of the distance to the slaughter facility and the cost of transportation, some lambs less than the desired weight were shipped to fill out a load.

As of the end of this second group of offspring slaughter (October, 2001), 227 lambs have been slaughtered. Because of light weights or tags lost during transportation, full carcass data is available on 197 head. This data is summarized in Table 1.

Table 1. Carcass data of feedlot lambs slaughtered in 2000 and 2001 by treatment group.

Group	N	Age in Days	Carc Wt	REA	Fat	Body Wall Th	Lbs Lean	Lbs Fat	# Lean/ Day	# Fat/ Day
FAT	107	224	62.7	2.24	.22	.88	37.8	15.6	.169	.070
LEAN	78	222	61.7	2.16	.21	.87	37.3	15.4	.168	.069
CONTROL	12	225	65.8	2.36	.20	.79	40.3	15.1	.172	.067

Table 2 summarizes the same data set by sire.

Table 2. Carcass data averages by sire for 2000 and 2001.

Sire	N	Age in Days	Carc Wt	REA	Fat	Body Wall Th	Lbs Lean	Lbs Fat	# Lean/ Day	# Fat/ Day
FAT 1	34	240	63.3	2.20	.21	.87	38.3	15.9	.160	.066
FAT 2	35	227	63.2	2.29	.20	.84	38.4	15.4	.169	.068
FAT 3	21	202	60.0	2.24	.24	.89	35.9	14.9	.177	.073
FAT 4	17	214	64.0	2.24	.27	.97	37.4	16.4	.175	.077
LEAN 1	21	229	62.4	2.21	.23	.90	37.7	15.8	.164	.069
LEAN 2	18	202	58.9	2.20	.19	.72	36.6	13.2	.181	.065
LEAN 3	38	227	62.7	2.11	.21	.93	37.2	16.4	.164	.072
CONTROL 1	5	230	72.3	2.44	.23	.82	43.8	17.3	.190	.075
CONTROL 2	7	212	62.7	2.33	.19	.79	38.3	14.4	.181	.068

Much of the differences between the F and L sired lambs which were demonstrated in the 2000 data for fat at the 12th rib and body wall has disappeared. Among the non-control sires, LEAN 2 had the highest lean per day and lowest fat per day averages for his offspring, however, he became unsound and was culled prior to the 2000 breeding season. Most of the differences in the averages in the 2000 data was due the offspring of LEAN 2. Furthermore, in an effort to increase the number of offspring born to each of the BIA selected rams, we culled two rams from each of the FAT and LEAN lines and, further, chose not to mate any ewes to the CONTROL rams in the fall of 2000. Lambs slaughtered in 2000 were slightly younger at slaughter, had slightly lighter carcasses, slightly larger REA, and slightly less body wall thickness than did the 2001 lambs (215 days vs 236; 61.9 pounds vs 63.5 pounds; 2.27 square inches vs 2.15 square inches; .84 inches vs .92 inches for 2000 vs 2001 respectively). Because only four rams had offspring represented in both years (FAT 1 and 2 and LEAN 1 and 3), year by ram interactions may have played a role in comparisons. The tradeoff is that more accurate evaluation of the four rams should result.

Within flock EPDs calculated for lean and fat tissue accretion per day of age based on offspring data were very similar for each of the four selected rams. These EDPs, when compared to the EPDs calculated from each ram's individual BIA data taken at six months of age, are summarized in Table 3.

Table 3. Within flock EPDs for lean and fat tissue accretion per day of age compared to individual ram BIAs.

Sire	Lean/Day Ind BIA	Fat/Day Ind BIA	Lean/Day Offspring	Fat/Day Offspring
LEAN 1	+0.0066	+0.0042	-0.00069	+0.00161
LEAN 3	+0.0128	-0.0065	-0.00094	+0.00505
FAT 1	+0.0094	+0.0069	-0.00496	-0.00057
FAT 2	+0.0117	+0.0079	+0.00368	+0.00071

The individual BIA based wfEPDs for LEAN 3 are what we were looking for: a **PLUS** value for lean tissue and a **NEGATIVE** value for fat tissue accretion. Unfortunately, the offspring calculated EPDs were the reverse of the desired. Comparison of pounds of retail product among the four rams revealed a range of just over a pound in offspring average. Since EPDs are calculated from the population average, if there is minimal difference within the population, there can be no great difference in the EPDs.

As a further analysis of the efficacy of BIA as a predictor of lean tissue for selecting replacement sires, we did a statistical procedure known as regression, regressing the offspring's carcass calculated pounds of retail product on the sire's BIA predicted pounds of retail product. Based on this data set, the correlation between the six-month BIA predicted pounds of retail product and the calculated pounds of retail product of their offspring carcasses was essentially zero. Again, in order to have statistical significance, there must be differences demonstrated somewhere in the data. The selected rams show little differences in tissue differentiation when the BIA analysis is done at six months of age. This lack of differences within the population is apparently what is frustrating our efforts at improvement.

EFFECTS OF PREPARTUM HIGH LINOLEIC SAFFLOWER SEED SUPPLEMENTATION FOR GESTATING EWES ON COLD TOLERANCE AND SURVIVABILITY OF LAMBS

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INTRODUCTION

Mortality of newborn lambs due to cold stress is a problem during winters and cold, wet springs. Cold and cold-induced starvation account for 50% of perinatal lamb deaths (Samson and Slee, 1981). Lambs produce 50 to 60 % of their heat through shivering and 40 to 50% through non-shivering thermogenesis (Alexander and Williams, 1968). Brown adipose tissue (BAT), present in most infant mammals, is the origin of the non-shivering portion of thermogenesis. Lambs are born with almost 100% BAT, unlike other species, such as humans and rats which are born with brown and white adipose tissue (WAT; Gemmel et al., 1972; Alexander and Bell, 1975). Uncoupling protein-1 (UCP1), also known as thermogenin, is specific to BAT and is the thermogenic agent.

Nutrition, particularly dietary fat, has been shown to influence BAT composition and activity. Research with steers (Cook et al., 1972) and lambs (Gibney and L'Estrange, 1975) utilizing feedstuffs high in linoleic acid resulted in increased linoleic acid content of specific brown fat stores. Brown adipose tissue relies on linoleic acid as a major fuel for heat production (Lammoglia et al., 1999a). Supplementation of sunflower and linseed oil (high in linoleic and linolenic acid) to rats increased the thermogenic capacity of BAT in by 75% and doubled the content of UCP1 (Nedergaard et al., 1983). Supplemental polyunsaturated fatty acids supplemented to rats increased thermogenic activity of BAT (Oudart et al., 1997).

Because cold stress is initiated as soon as the lamb leaves the temperature controlled uterine environment upon parturition, the optimal period for enhancing the thermogenic potential of the lamb would be prior to parturition. However, few studies have examined the effects supplemental fat to the gestating ewes on the neonatal lamb. Budge et al. (2000) demonstrated ewes well-fed (150% metabolic requirement) during the final 65 d of gestation had lambs with 22% more UCP1 abundance and twice the thermogenic activity in BAT as lambs from ewes fed at 100% requirement. It is not known whether such increases would result in a more cold tolerant lamb. Lammoglia et al. (1999a,b) fed high linoleic safflower seeds to heifers during the last third of gestation and calves were better able maintain body temperature when exposed to cold compared to calves from dams fed conventional supplements.

High linoleic safflower seeds may be an feasible dietary source of linoleic acid for livestock. Seeds from the high linoleic varieties can contain up to 80% linoleic acid. In addition, the seed, because of the high oil content, is a high energy feed and a good source of rumen degradable protein, making it a good source of supplemental nutrients. The objectives of this study were to determine if feeding high linoleic safflower seed as a fat source to gestating ewes increases the cold tolerance, overall survivability, and performance of lambs.

PROCEDURES

These projects were conducted at the North Dakota State University Hettinger Research Extension Center located just west of Hettinger, ND. Average high and low temperature during supplementation were 0 and -12.8°C (yr 1) and -0.7 and -12.2°C (yr 2), respectively. During lambing, average high and low temperatures for yr 1 were 7.9 and -5.6°C and -6.8 and -17.8°C for yr 2, respectively.

Approximately 45 d prior to anticipated lambing date, 122 (yr 1; 75.8 ± 7.6 kg initial weight) and 112 (yr 2; 75.8 ± 7.6 kg initial weight) gestating ewes were allotted randomly to one of two dietary

treatments (4 pens/treatment). Pregnancies were verified with real-time ultrasound. Ewes were fed diets formulated to contain either 1.9 (low fat; LF) or 4.6% (high fat; HF) dietary fat. Diets were delivered via a 10 ft self-feeder with feed access on both sides as a total mixed ration and diets were calculated to be isocaloric and isonitrogenous. In addition to finely chopped alfalfa hay, rolled safflower seeds (32% fat; 80% linoleic acid) were supplemented in HF, while solvent extracted safflower meal was used as protein source in LF supplement. Energy was balanced in LF with corn (Table1). In yr 1 all pens were offered equal amounts of feed. Ewes were allowed to consume free choice trace mineralized salt block. Animals were housed in 10 X 100 ft pens with access to a 10 X 30 ft covered barn.

At the onset and conclusion of supplementation, ewes were weighed and body condition was scored (BCS) using a five point scoring system (1 = emaciated, 5 = obese). Upon lambing, birth weights were recorded. Lamb mortality was recorded and separated by cause: born dead, pneumonia, or starvation. Lambing rates were calculated by dividing number lambs born per pen by number ewes per pen. In calculating mortality, number lambs died (total or of a certain cause) per pen were divided by total lambs born per pen. Surviving lambs were weighed at weaning. Lambs weaned per ewe was calculated by dividing number of lambs surviving until weaning per pen by number of ewes per pen. Sum of weaning weight of lambs per pen were divided by number of ewes per pen to find lbs weaned per ewe.

Table 1. Diet and nutrient composition of gestating ewes fed low or high fat diets (DM basis).

	Treatment	
Ingredient, %	LF	HF
Alfalfa hay, ground	78.3	81.6
Corn, dry rolled	14.0	5.5
Safflower meal, solvent extruded	6.6	2.5
Molasses, dry	1.1	0.4
Safflower seeds, rolled	—	10.0
Nutrient composition, %		
Dry matter	81.35	81.72
Organic matter	91.74	91.12
Crude protein	17.45	17.78
Ca	0.96	1.18
P	0.38	0.26
Fat	2.8	5.7
ME[a], Mcal/kg	2.24	2.27

[a]Metabolizable energy; calculated.

RESULTS AND DISCUSSION

Ewe Performance

Data was combined across years for all variables. Ewes consumed an average of 3.22 and 2.60 kg dry matter daily per year respectively. Body condition at the onset (3.66 ± 0.03; $P = 0.45$; Table 2)

and conclusion (3.92 ± 0.02; $P = 0.46$) of supplementation was similar for LF and HF fed ewes. Differences in initial (78.7 ± 0.6; $P = 0.25$) and final BW (95.0 ± 0.9; $P = 0.22$) were not detected. Lack of difference in body weight and condition were expected as both diets provided equal amounts of energy. These results are in agreement with Lammoglia et al. (1999a) feeding isocaloric and isonitrogenous diets including safflower seeds to heifers. Three treatment diets containing different types of oilseeds did not cause differences in heifer body condition or weight over a control diet similar in energy and protein concentration (Bellows et al., 1999). Similar results have been seen with mature cows (Lammoglia et al., 1997). De Fries et al. (1998), however, observed increased body condition with no change in BW of cows fed isocaloric and isonitrogenous diets containing rice bran following calving.

Table 2. Effect of safflower supplementation on ewe performance.

	Treatment			
Item	LF	HF	SEM[a]	P[b]
Weight, lb				
Initial	174.0	171.6	1.3	0.25
Final	210.5	206.8	2.0	0.22
Body condition score[c]				
Initial	3.68	3.65	0.03	0.45
Final	3.93	3.91	0.02	0.46

[a]Standard error of the mean; n = 4.
[b]Probability of a greater F statistic.
[c]1=emaciated, 5=obese

Lamb Birth Weight, Mortality, and Weaning Performance

Birth weights of lambs were not different ($P = 0.24$; Table 3) probably due to the energy equality in the dietary treatments. Additional energy provided to the dam during the last trimester has been hypothesized to stimulate greater fetal growth. The current results agree with supplemental fat studies in beef females (Bellows et al., 1999; Espinoza et al., 1995). Lambs from HF dams had higher survivabilities ($P = 0.03$). There was no difference in numbers of lambs born dead; however, more ($P = 0.03$) lambs from LF dams died due to starvation and tended to die from pneumonia ($P = 0.07$).

Lambs are most susceptible to hypothermia from birth to five hours of age and again 12 to 36 hours after birth (Eales et al., 1982). Stott and Slee (1985) state, "A viable lamb must, therefore, be vigorously homeothermic at birth and possess sufficient energy reserves." Therefore, methods imposed during fetal development to increase the thermogenic capacity and energy reserves of the lamb at the onset of parturition could decrease mortality due to cold stress in the first 36 hours after birth. The present study showed lambs had greater survivabilities when dams had been fed a high linoleic safflower during the last 45 days of gestation.

There was no difference in weaning weights across treatments ($P = 0.18$; Table 3). Bellows et al. (1999) saw a tendency of fat supplementation to heifers during the last 65 d of pregnancy to increase WW of calves. Calves from HF supplemented dams have had increased WW (Espinoza et al., 1995); however, fat supplementation was continued after parturition and during lactation. Due to higher mortality at parturition, number of lambs weaned per ewe was lower for the LF treatment ($P = 0.02$).

Table 3. Influence of dietary treatment of dam on lamb birth weight, mortality, and weaning weight.

	Treatment			
Item	LF	HF	SEM[a]	P^b
Birth weight, kg	5.7	5.6	0.09	0.24
Lambs per ewe	1.63	1.76	0.06	0.13
Mortality, % of total lambs born	21.68	11.57	2.94	0.03
Born dead, % of total lambs born	3.24	5.78	2.18	0.43
Starvation, % of total lambs born	15.38	5.79	2.79	0.03
Pneumonia, % of total lambs born	1.74	0.00	0.63	0.07
Weaning weight, kg	19.2	17.8	0.68	0.18
Lambs weaned per ewe	1.17	1.47	0.04	0.02
Lamb weight weaned per ewe, kg	21.4	24.8	1.5	0.12

[a]Standard error of the mean; n = 4.
[b]Probability of a greater F statistic.

CONCLUSIONS

Results from this experiment suggest feeding high linoleic safflower seed to ewes during the last 45 days of gestation increases lamb survivability at parturition. Increasing number of lambs born live and weaned suggests an economic benefit from supplementation. Further research is necessary in eliciting the mechanism by which survival is increased and identifying types of fat sources which cause such a response.

LITERATURE CITED

Alexander, G. and A. W. Bell. 1975. Quantity and calculated oxygen consumption during summit metabolism of brown adipose tissue in newborn lambs. Biol. Neonate. 26:214-220.

Alexander, G. and D. Williams. 1968. Shivering and non-shivering thermogenesis during summit metabolism in young lambs. J. Physiol. 198:251-276.

Bellows, R. A., D. D. Simms, E. E. Grings, D. A. Phelps, S. E. Bellows, N. R. Bellows, R. E. Short, R. N. Funston, and T. W. Geary. 1999. Effects of feeding supplemental fat during gestation on reproduction in primiparous beef heifers. J. Anim. Sci. 77 (Suppl. 1):236 (Abstr.).

Budge, H., J. Bispham, J. Dandrea, E. Evans, L. Heasman, P. M. Ingleton, C. Sullivan, V. Wilson, T. Stephenson, and M. E. Symonds. 2000. Pediatr. Res. Effect of maternal nutrition on brown adipose tissue and its prolactin receptor status in the fetal lamb. 47:781-786.

Cook, L. J., T. W. Scott, G. J. Faichney, and H. L. Davies. 1972. Fatty acid interrelationships in plasma, live, muscle, and adipose tissues of cattle fed sunflower oil protected from ruminal hydrogenation. Lipids. 7:83-89.

De Fries, C. A., D. A. Neuendorff, and R. D. Randel. 1998. Fat supplementation influences postpartum reproductive performance in Brahman cows. J. Anim. Sci. 76:864-870.

Eales, F. A., J. S. Gilmour, R. M. Barlow, and J. Small. 1982. Causes of hypothermia in 89 lambs. Vet. Rec. 110:118-120.

Espinoza, J. L., J. A. Ramirez-Godinez, J. A. Jimenez, and A. Flores. 1995. Effects of calcium soaps of fatty acids on postpartum reproductive activity in beef cow and growth of calves. J. Anim. Sci. 73:2888-2892.

Gemmell, R. T., A. W. Bell, and G. Alexander. 1972. Morphology of adipose cells in lambs at birth and during the subsequent transition of brown to white adipose tissue in cold and warm conditions. J. Anat. 133:134-164.

Gibney, M. J. and J. L. L'Estrange. 1975. Effects of dietary unsaturated fat and of protein source on melting point and fatty acid compotition of lamb fat. J. Agri. Sci. 84:291-296.

Lammoglia, M.A., R. A. Bellows, E. E. Grings, and J. W. Bergman. 1999a. Effects of prepartum supplementary fat and muscle hypertrophy genotype on cold tolerance in newborn calves. J. Anim. Sci. 77:2227-2233.

Lammoglia, M. A., R. A. Bellows, E. E. Grings, J. W. Bergman, R. E. Short, and M. D. MacNeil. 1999b. Effects of feeding beef females supplemental fat during gestation on cold tolerance in newborn calves. J. Anim. Sci. 77:824-834.

Lammoglia, M. A., S. T. Willard, D. M. Hallford, and R. D. Randel. 1997. Effects of dietary fat on follicular development and circulating concentrates of lipids, insulin, progesterone, estradiol 17β, 13, 14-dihydro-15-keto-prostaglandin $F_{2\alpha}$, and growth hormone in estrous cyclic Brahman cows. J. Anim. Sci. 75:1591-1600.

Nedergaard, J., W. Becker, and B. Cannon. 1983. Effects of dietary essential fatty acids on active thermogenin content in rat brown adipose tissue. J. Nutr. 113:1717-1724.

Oudart, H., R. Groscolas, C. Calgari, M. Nibbelink, C. Leray, Y. Le Maho, and A. Malan. 1997. Brown fat thermogenesis in rats fed high-fat diets enriched with n-3 polyunsaturated fatty acids. Inter. J. Obes. 21:955-962.

Samson, D. E. and J. Slee. 1981. Factors affecting resistance to induced body cooling in newborn lambs of 10 breeds. Anim. Prod. 33:59-65.

Stott, A. W., and J. Slee. 1985. The effect of environmental temperature during pregnancy on thermoregulation in the newborn lamb. Anim. Prod. 41:341-347.

Level of rumen undegradable protein (RUP) in high-grain diets fed to feedlot lambs
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Introduction

Rumen undegradable protein (RUP) is protein that bypasses the rumen. Rumen undegradable protein flows to the small intestine where digestion takes place. Rumen degradable protein (RDP) is protein that is broken down by ruminal microorganisms. Loe et al. (2000) evaluated the addition of RDP and RUP to corn-based lamb finishing diets. Treatments with added RUP contained 7.3% RUP. Lambs fed additional RUP had heavier final weights, gained more rapidly, and were more efficient compared with RDP fed lambs. Loe et al. (2001) conducted research to determine the optimum level of RDP in lamb finishing diets. Feeding levels between 6.1 and 11.0% RDP did not affect gain or feed efficiency. They concluded that the optimal level of RDP does not appear to be above 6.1% in lambs with the ability to gain at least 1.06 lb/day. Little research has been conducted on RUP in lamb finishing diets. The objective of this study was to determine the optimum level of RUP in lamb finishing diets.

Materials and Methods

Seventy-four crossbred ram lambs (85.4 ± .83 lb initial weight) were fed for 74 d days to determine the optimal level of RUP in corn-based finishing lamb diets. Lambs were housed at the NDSU Animal Research Center barns. The lambs were blocked by weight and allotted randomly to dietary treatments (5 pens/treatment). Each pen had access to an outdoor and indoor run and fresh water.

Diets consisted of corn, beet pulp pellets, molasses, soybean meal, urea, feathermeal, bloodmeal, and supplement (Table 1). Diets were offered ad libitum once daily. Treatments contained 6, 7, 8, or 9% RUP (dry matter basis). Rumen undegradable protein sources were feathermeal and bloodmeal combinations. Level of RDP was held constant at 6.9% in all treatments.

Three day weights were taken at the beginning and end of the trial to measure average daily gain. Unconsumed feed was removed from the bunks and weighed weekly to measure dry matter intake. Carcass data was collected at slaughter. Hot carcass weight, fat thickness, rib-eye area, bodywall thickness, marbling, flank streaking, leg score, and conformation score were taken.

Results

No treatment affects on performance occurred (Table 2). Rib-eye area increased linearly (P = 0.06) with increasing level of RUP. There were no other affects on carcass characteristics (Table 3) with respect to treatment.

Conclusions

Lack of treatment effects may indicate that lambs on finishing diets do not need more than 6% RUP. However, a previous study indicates improved performance with lambs fed 7.3% RUP versus 5.9%.

The lack of treatment effects in this study may be due to poor lamb performance. In this study, lambs gained only 0.66 lb/day. In a previous study (Loe et al., 2000), lambs fed RUP at 7.3%

gained .87 lb/day. In another study (Loe et al., 2001), lambs fed 7.3% RUP with varying levels of RDP gained 1.07 lb/day.

Table 1. Formulated dietary treatments (%, DM Basis)				
	Level of rumen undegradable protein, (%, DM Basis)			
Item	6	7	8	9
Corn	75.43	74.28	73.13	71.99
Beet pulp pellets	12.50	12.50	12.50	12.50
Molasses	5.00	5.00	5.00	5.00
SBM	1.99	1.33	0.66	0.00
Urea	0.22	0.15	0.07	0.00
Feathermeal	1.51	3.02	4.53	6.04
Bloodmeal	0.38	0.76	1.13	1.51
Supplement	2.97	2.97	2.97	2.97
Protein				
Crude	12.90	13.90	14.90	15.90
Rumen degradable	6.90	6.90	6.90	6.90
Rumen undegradable	6.00	7.00	8.00	9.00
Metabolizable	7.66	8.47	9.28	10.08

Table 2. Effect of treatment on performance					
	Level of rumen undegradable protein,(%, DM Basis)				
Item	6	7	8	9	Standard Error
Weight, lb					
Initial	85.3	84.7	86.1	85.5	0.8
Final	131.3	134.3	136.7	134.8	2.5
Dry Matter Intake					
lb/day	3.01	3.08	3.30	3.19	0.10
% of body weight	2.80	2.82	2.99	2.90	0.08
Average daily gain, lb					
live wt basis	0.62	0.67	0.68	0.67	0.04
carcass basis[a]	0.65	0.68	0.67	0.68	0.04
Gain:Feed	0.206	0.217	0.206	0.210	0.007
Feed:Gain	4.85	4.60	4.85	4.76	---

[a] Based on hot carcass weight and 50% dress

Table 3. Effect of treatment on carcass characteristics					
	Level of rumen undegradable protein, (DM Basis)				
Item	6	7	8	9	Standard Error
Hot carcass weight, lb	66.55	67.49	68.01	67.90	1.42
Fat thickness, in	0.17	0.17	0.17	0.19	0.02
Rib-eye area, in^2 [a]	2.35	2.49	2.49	2.53	0.06
Bodywall thickness, in	0.91	0.93	0.86	0.90	0.04
Marbling	375	389	377	359	17
Flank streaking	369	339	350	337	14
Yield grade	2.08	2.12	2.12	2.30	0.18
Leg score	11.12	11.20	11.50	11.22	0.21
Conformation score	11.07	11.20	11.25	11.22	0.22

[a] Linear response to treatment (P = 0.06)

[b] Marbling score of 300 = slight

[c] Score of 11 = low choice

Evaluation of Katahdin and Wiltshire Horn (Hair Sheep) Breeds: Progress Report

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Introduction

A growing interest in hair sheep has, in part, been initiated by a decline in value in wool. Many sheep producers have found that shearing costs are unable to be offset by income from wool. Some of the hair sheep seem to have potential as effective biological weed (leafy spurge) controls. Most of these breeds have evolved and are produced in forage based, relatively low input management programs which likewise seem to appeal to those with limited sheep backgrounds and experience.

Procedures

Background and information on the two breeds being evaluated, Katahdin and Wiltshire Horn has been presented in previous Western Dakota Sheep Day Reports, Moore, et. al. 2001. An additional finishing trial was conducted to evaluate these sheep under feedlot conditions and to obtain additional carcass data to add to that obtained last year.

A group of Katahadin (K), Wiltshire Horn (W) and Columbia and Hampshire (CH) sired lambs out of commercial ewes were placed on a finishing trial for a 78 day duration. A 16 percent protein complete mixed ration containing 12 per cent alfalfa pellets as a roughage source was fed free choice.

Results

Results of the finishing trial are listed in table 1.

Table 1. Finishing Data

Sire Group	K	W	CH
ADG (lbs)	.542	.732	.670
Feed intake/d (lbs)	4.19	4.94	4.15
Feed/Gain	7.72	6.75	6.19
Dry Matter/Gain (lbs)	6.85	5.99	5.49

The patterns of growth and efficiency did not show any particular similarities to those obtained in the year 2000. One replicate of Katahdin sired lambs did not gain as well as the others which accounts for the lower gain figures for them. All values were lower than in the previous year and can perhaps be explained by an extremely hot summer period when the lambs were on feed.

Carcass data are presented in table 2.

Table 2. Carcass data

Sire Group	K	W	CH
Hot Carcass Weight (lbs)	60.13	63.15	61.00
REA (in. sq.)	2.26	2.28	2.33
Conformation Score[1]	10.07	10.32	10.26
Lean Color[2]	2.92	2.70	2.89
Fat (in.)	.16	.20	.16
Body Wall Thickness (in.)	.83	.88	.75
Percent BCTRC[3]	42.37	39.99	43.64

[1] $10 = Ch^{-}$, $11 = Ch^{0}$, $12 = Ch^{+}$
[2] 3 = ideal lamb color, reddish pink
[3] Percent boneless, closely trimmed retail cuts

Carcass data do not indicated large differences except that the W sired lambs were the fattest and had the highest body wall thickness which contributed to them having the lowest per cent BCTRC. In general the carcass data indicates that these hair breeds of sheep can sire lambs that can be very acceptable to the trade and are well above the averages for the industry.

Conclusions

- Although not previously stated, it continues to be obvious that the lambs sired by hair rams exhibit an extreme amount heterosis (as expected) with their vigor and livability at birth.
- Gains and efficiencies continue to look acceptable for lambs of these genetic backgrounds
- Rams of hair breeding can sire market lambs that will produce carcasses very acceptable to the industry trade.

Progress and Future Plans

A purebred flock of Katahdin sheep has been established at the NDSU Sheep Barn. Ewes and rams were purchased from the Lovelace flock in Missouri and the Fortmeyer flock in Kansas. Interest in these sheep continues to run quite high and it is hoped that this flock can serve as a resource of breeding stock as well as information about the merits of these sheep.

References

Moore, B.L., P.T. Berg, R. Haugen, W. Limesand, D.A. Redmer, A. Grazul-Bilska. 2001. Evaluation of Katahdin and Wiltshire Horn (Hair Sheep Breeds): Their effectiveness in low input management schemes. 42nd Western Dakota Sheep Day Report. Hettinger, ND. pp72-76.

Wildeus, S. 1997. Hair sheep genetic resources and their contribution to diversified small ruminant production in the United States. J. Animal Sci. 75:630-640.

A Preliminary Report on Early Embryonic Development Following Oocyte Vitrification and In Vitro Fertilization in Sheep

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INTRODUCTION

Interest in oocyte cryopreservation has recently increased with the growing importance of *in vitro* embryo production, nuclear transfer, and gene banking (Gordon, 1997; Andrus et al., 2000). A relatively recent approach to achieve rapid freezing without the use of expensive freezing devices is called vitrification. Vitrification is defined as the physical process by which a highly concentrated solution of cryoprotectant solidifies during cooling without formation of ice crystals (Rall, 1987). Oocyte vitrification has been developed using mouse embryos (Rall et al., 1985). Recently, vitrification has also been attempted in the cow (Fuku et al., 1992; Vajta et al., 1998; Hurtt et al., 2000; Papis et al., 2000), pig (Isachenko et al., 1998), buffalo (Dhali, et al., 2000), and horse (Hurtt et al., 2000). The advantages of vitrification technology when compared with slow-rate freezing are the low price of equipment, the simplicity of the procedure, and the short time required (Palasz et al., 1996).

Despite the efforts of numerous research groups, successful cryopreservation of oocytes remains a difficult task (Niemann, 1991; Okatay et al., 1998). A limited number of experiments have reported blastocyst development and subsequent calf development from oocytes that have gone through the vitrification process (Fuku et al., 1992; Vajta et al., 1998). This lack of success presents an obstacle to planning and organizing of experiments (Ana et al., 2001).

The aim of this experiment was to evaluate early embryonic development following vitrification of oocytes at the germinal vesicle (GV) stage and *in vitro* fertilization (IVF) in sheep treated with follicle stimulating hormone (FSH).

MATERIALS AND METHODS

Ewes of mixed breeds (n = 5) received exogenous FSH on days 13 and 14 of the estrous cycle (Stenbak et al., 2001). Ovaries were surgically removed by ovariectomy (Reynolds et al., 1998) and placed in phosphate-buffer saline (PBS) containing penicillin/streptomycin (Gibco, Gaithesburg, MD) at 39° C (Stenbak et al., 2001). The number of visible follicles on each ovary was counted and oocytes were collected. Oocytes were then evaluated based on morphology and categorized as healthy or atretic according to Thompson et al. (1995).

All oocytes were washed three times in maturation medium (Stenbak et al, 2001). Oocytes were incubated in stabilized maturation medium (incubated overnight under oil at 39° C in 5% CO_2 and 95% air) containing 10 ng/ml of epidermal growth factor (EGF; Sigma). Dose of EGF was chosen on the basis of previously published experiments (Guler et al 2000; Grazul-Bilska et al., 2001).

After four hours, oocytes from each ewe were divided into two treatment groups: control (CON) and vitrification (VIT). For CON group, oocytes were matured *in vitro* for additional 20 hours. Oocytes in the VIT group were vitrified, thawed, and then matured for additional 20 hours. There were two steps in the vitrification process. First, oocytes were placed in holding medium [TCM-Hepes supplemented with 20 % fetal bovine serum (FBS) containing 10 % ethylene glycol (EG) and 10 % dimethyl sulfoxide (DMSO)] at 38°C for 45 seconds (Vajta et al., 1998). Second, oocytes were placed to holding medium containing 20 % EG, 20 % DMSO and 0.5 M sucrose at 38 °C for 25 seconds. Oocytes were then placed directly in liquid nitrogen (LN_2, - 196 °C) for 1 hr followed by the thawing process.

The thawing process consisted of three steps. First, oocytes were placed in holding medium containing 0.25 M sucrose for 1 min. Second, oocytes were placed in a holding medium containing 0.15 M sucrose for 5 min. Third, oocytes were placed in holding medium for two, 5-minute intervals. Subsequently, oocytes continued the maturation process and were then subjected to IVF and culture as described previously (Stenbak et al. 2001; Grazul-Bilska et al., 2001).

All data are reported as means ± standard error (SEM). Data were analyzed by using the general linear models (GLM) procedure of the Statistical Analysis System (SAS, 1985) with the vitrification process as the main effect. In addition, data for the number of oocytes cleaved, morula, and blastocysts were analyzed by Chi-square (Spiegel, 1961).

RESULTS

The number of visible follicles was 30.4 ± 1.8/ewe, the number of recovered oocytes was 31.2 ± 2/ewe, and the number of healthy oocytes was 22.4 ± 2.3/ewe.

The total number of oocytes used for IVM was 150, the CON group consisted of 71 oocytes, and the VIT group consisted of 79 oocytes. Following maturation, cumulus cells were much more expanded in the CON than VIT group. Some oocytes were damaged in the VIT group.

Table 1 shows the number of oocytes used for IVF, oocytes fertilized, and the number of morula and blastocysts in the CON and VIT group.

Table 1. Number of oocytes used for IVF, oocytes fertilized, morulas and blastocysts of CON and VIT groups.

Treatment	No.of oocytes used for IVF		No. of fertilized oocytes (%)		No. of non-fertilized oocytes (%)		No. of morula (%) on day 6		No. of blastocyst (%) on day 8	
	Total	Per ewe	Total	Per ewe	Total	Per ewe	Total	Per ewe	Total	Per ewe
Control (CON)	71	17.8 ±4	27* (38*)	6.8±2.6	44 (62)	11±5.4	6 (8.5)	1.5±0.7	2 (3)	0.5±0.2
Vitrification (VIT)	79	19.8±4.8	6 (7.5)	1.5±2.6	73 (92.5)	18.3±5.4	2 (2.5)	0.5±0.7	0 (0)	0

* Values (means ± SEM) differ within a column; $P<0.05$

Control oocytes achieved a greater ($P<0.05$) fertilization rate than oocytes from the VIT group. Total number of fertilized oocytes was also greater ($P<0.05$) in CON than VIT group. However, no differences ($P>0.1$) were observed between CON and VIT groups in morula and blastocyst development following fertilization.

DISCUSSION

Data of this experiment demonstrated that the vitrification procedure could be applied to cryopreserve oocytes before fertilization since several embryos developed after vitrification and IVF. However, fertilization rates of vitrified oocytes were significantly lower than control oocytes. Therefore, future studies will need to focus on improving the process of vitrification for subsequent fertilization and early production procedures in sheep.

Vitrification has been used to cryopreserve oocytes in several species, including mice (Rall and Fahy, 1985; Ko and Threlfall, 1988), cow (Fuku et al., 1992; Martino et al., 1996; Vajta et al., 1998; Hurtt et al., 2000), pig (Isachenko et al., 1998), horse (Hurtt et al., 2000) and buffalo (Dhali et al., 2000). However, the efficiency of this procedure is very low. Vitrification diminishes the rates of maturation, fertilization and blastocyst formation, when compared to slow-rate freezing techniques and/or no freezing conditions (Fuku et al., 1992, 1995; 30% Martino et al., 1996; Isachenko et al., 1998;Vajta et al., 1998; Dhali et al., 2000; Hurtt et al., 2000).

In our experiment, the fertilization rate of non-vitrified oocytes was 38% and vitrified oocytes 7.5%. Additional data for vitrified ovine oocytes are not available at present, but for non-vitrified oocytes the rates of fertilization are usually in the range 60-85% (see Grazul-Bilska et al., 2001 and Stenbak et al., 2001). Relatively high fertilization rates (45-75%) of vitrified oocytes were reported for cows (Vajta et al., 1998; Papis et al., 2000). However, these fertilization rates were lower than achieved for non-vitrified oocytes (72-91%; Vajta et al., 1998; Papis et al., 2000). Others reported much lower rates of fertilization for bovine vitrified oocytes (0.8-7%, Fuku et al., 1992, 1995; 30%, Martino et al., 1996). Bovine vitrified and then fertilized oocytes were able to develop to blastocyst stage (Papis et al., 2000; Vajta et al., 1998; Martino et al., 1996). Interestingly, healthy calves were born following transfer of embryos developed after vitrification of oocytes (Fuku et al., 1992; Vajta et al., 1998).

High fertilization rates for bovine vitrified oocytes were achieved by introducing several modifications to the simple vitrification procedure (Papis et al., 2000). Therefore, these modified protocols will be used in future experiments to improve the vitrification procedure for ovine oocytes.

In summary, the present data demonstrated that vitrification procedures have a potential to be used to cryopreserve ovine oocytes. Future experiments have the potential to establish an efficient vitrification method for ovine oocytes. Vitrification can be applied to make oocyte banks for storage of gametes from genetically superior ewes.

ACKNOWLEDGMENTS

Supported in part by Hatch Project ND 01705 and ND SBARE.

LITERATURE CITED

Ana, C., Carmen, R., Sandro, G., Amparo, R., Antonio, P., and Jose, R. 2001. Use of fluorescence in situ hybridization to assess the chromosomal status of embryos obtained from cryopreserved oocytes. Fertil. Steril. 75: 354-360.

Andrus, D., Yunping, D., Shie, J., and Xiangzhong, Y. 2000. High developmental rates of vitrified bovine oocytes following parthenogenetic activation, *in vitro* fertilization, and somatic cell nuclear transfer. Biol. Reprod. 63: 513-518.

Dhali, A., Manik, R.S., Das, S.K., Singla, S.K., and Palta, P. 2000. Post-vitrification survival and in vitro maturation rate of buffalo (Bubalus bubalis) oocytes: effect of ethylene glycol concentration and exposure time. Anim. Reprod. Sci. 63: 159-165.

Fuku, E., Kojima, T., Shioya, Y., Marcus, G.J., and Downey, B.R. 1992. In vitro fertilization and development of frozen-thawed bovine oocytes. Cryobiology 29: 485-492.

Fuku, E., Xia, L., and Downey, B.R. Ultrastructural changes in bovine oocytes cryopreserved by vitrification. Cryobiology 1995; 32:139-56.

Gordon, I. 1997. Controlled reproduction in sheep and goats. CAB International

Grazul-Bilska, A.T., Choi, J.T., Bilski, J.J., Weigl, R.M, Kraft, K.C., Reynolds, L.P., and Redmer, D.A., 2001. Effects of epidermal growth factor (EGF) on oocyte maturation, *in vitro* fertilization (IVF) and blastocyst formation in ewes treated with follicle stimulation hormone (FSH). Western Dakota Sheep Day, Hettinger, ND, Report 42: 5-11.

Guler, A., Poulin, N., Mermillod, P., Terqui, M., and Cognie, Y. 2000. Effect of growth factors, EGF and IGF-1, and estradiol on *in vitro* maturation of sheep oocytes. Theriogenology 54: 209-218.

Hurtt, A.E., Landim-Alvarenga, F., Seidel, G.E. Jr., and Squires, E.L. 2000. Vitrification of immature and mature equine and bovine oocytes in an ethylene glycol, ficoll and sucrose solution using open-pulled straws. Theriogenology 54: 119-128.

Isachenko, V., Soler, C., Isachenko E., Perez-Sanchez, F., and Grishchenko, V. 1998. Vitrification of immature porcine oocytes: effects of lipid droplets, temperature, cytoskeleton, and addition and removal of cryoprotectant. Cryobiology 36: 250-253.

Ko, Y., and Trelfall, W.R. 1998. The effects of 1,2-propanediol as a cryoprotectant on the freezing of mouse oocytes. Theriogenology 29: 987-995.

Martino, A., Songsasen, N., Leibo, S.P. Development into blastocysts of bovine oocytes cryopreserved by ultra-rapid cooling. Biol. Reprod. 54: 1059-1069.

Niemann, H. 1991. Cryopreservation of ova and embryos from livestock: current status and research needs. Theriogenology 35: 109-124.

Okatay, K., Newton, H., Aubard, Y., Salha, O., and Gosden, R., 1998. Cryopreservation of immature human oocytes and ovarian tissue: an emerging technology? Fertil. Steril. 69: 1-7

Palasz, A.T., and Mapletoft, R.J. 1996. Cryopreservation of mammalian embryos: recent advances. Biotechnol. Adv. 14: 127-149.

Papis, K., Shimizu, M., and Izaike, Y. 2000. Factors affecting the survivability of bovine oocytes vitrified in droplets. Theriogenology 54: 651-658.

Rall, W.F., and Fahy, G.M. 1985. Vitrification: a new approach to embryo cryopreservation. Theriogenology 23: 220.

Rall, W.F. 1987. Factors affecting the survival of mouse embryos cryopreserved by vitrification. Cryobiology 24: 387-402.

Reynolds, L.P., Kirsch, J.D., Kraft, K.C., Knutson, D.L., McClaflin, W.J. and Redmer, D.A. 1998. Time-course of the uterine response to estradiol-17 β in ovariectomized ewes: Uterine growth and microvascular development. Biol. Reprod. 59: 606-612.

SAS. User's Guide, Statistics, 5th Edn. 1985. Statistical Analysis System Inst., Cary, NC.

Spiegel, M.R. 1961. Theory and Problems of Statistics. Schaum's outline series, McGraw-Hill Book Co. New York, NY.

Stenbak, T.K., Erickson, A.S., Berginski, H., Toutges, M.J., Redmer, D.A., Reynolds, L.P., and Grazul-Bilska, A. T. 2001. Effects of follicle stimulating hormone (FSH) on follicular development, oocyte retrieval, and *in vitro* fertilization (IVF) in ewes during breeding season and seasonal anestrus. Theriogenology 56: 51-64.

Thompson, J.G., Gardner, D.K., Pugh, P.A., McMillan, W.H., and Tervit, H.R. 1995. Lamb birth weight is affected by culture system utilized during *in vitro* Pre-elongation development of ovine embryos. Biol. Reprod. 53: 1385-1391.

Vajta, G., Holm, P., Kuwayma, M., Booth, P.J., Jacobsen, H., Greve, T., and Callesen, H. 1998. Open pulled straw (OPS) vitrification: a new way to reduce cryoinjuries of bovine ova and embryos. Mol. Reprod. Dev. 51: 53-58.

A Preliminary Report of Laparoscopic Oocyte Collection in Ewe Lambs and Aged Ewes Treated with Follicle Stimulating Hormone During the Breeding Season;

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INTRODUCTION

The potential application of in vitro embryo production (IVP) technologies partially depends on the development of reliable, repeatable and efficient techniques for recovery of oocytes from genetically valuable animals. While oocyte collection from living animals has been widely used in the bovine species, its exploitation in sheep has been limited (Galli et al., 2001). Most work reported in sheep has been accomplished with slaughterhouse-derived oocytes (Naitana et al., 1992; Guler et al., 2000; Gordon, 1997; Cognie, 1999).

However, Snyder and Dukelow (1974) pioneered laparoscopic oocyte collection in sheep, and this technique was further improved in recent years (Tervit et al., 1992; Baldassarre et al., 1994; Earl and Kotaras, 1994; Stangl et al., 1999). Laparoscopic oocyte collection is an effective and minimally invasive technique, which offers the possibility of repeated ovum pick-up and allows for repeated production of embryos from a single donor ewe. Nor does this technique cause permanent damage to the donor ewe's reproductive health. In addition, the donor ewe can be in almost any physiological status and still be suitable for oocyte recovery (Gordon, 1997).

The objective of this study was to determine if laparoscopic oocyte collection can be used as an efficient and effective technique for retrieving oocytes from ewe lambs and aged ewes treated with follicle stimulating hormone (FSH) during the breeding season.

MATERIALS AND METHODS

Animals, Experimental Design, and Oocyte Collection

Crossbred Columbia x Hampshire ewe lambs and Rambouillet x Targhee western range ewes aged (2-6years old) were used in this study (n=6/age group). All oocyte collections were performed over a period of three weeks during the normal breeding season (November and December, 2001). Only ewes having a normal estrous cycle (15-17 days; determined using vasectomised rams) were used. For induction of superovulation, the ewes were given intra-muscular (i.m.) injections of FSH-P (Sioux Biochemical, Sioux Center, IA) twice daily on days 13 and 14 of the estrous cycle before oocyte collection (Stenbak et al., 2001).

All ewes were kept off feed and water for a period of 24 h before oocyte collection. The collection was performed under sedation using Rompun (0.1 mg/kg i.m.) and Ketaset (1.25 μg/kg, i.m.; Gourley and Riese, 1990). Laparoscopy was performed as previously described Gourley and Riese, 1990). The position of the ovaries was viewed and the number of follicles on each ovary was counted. All visible follicles were aspirated with an 18-gauge double lumen

ovum pick-up needle (Cook Veterinary Products, Spencer, Inc.) using oocyte collection media containing heparin (Stenbak et al., 2001).

After oocyte collection, the contents of the collection vial were transferred to a petri dish for observation under a stereomicroscope. Recovered oocytes were transferred into a petri dish with fresh collection medium without heparin (Stenbak et al., 2001) and the number of collected oocytes was determined.

Statistical Analysis

Numbers of follicles observed and aspirated, number of oocytes collected, and percentage of oocytes collected were analyzed by using the general linear models procedure of the Statistical Analysis System (User's Guide, 1985).

RESULTS

Table 1 presents the number of follicles observed, number of follicles aspirated, number of oocytes collected and recovery rate of oocytes for ewe lambs and aged ewes.

Table 1. Number of observed follicles, aspirated follicles, oocytes collected and the percentage of oocytes collected for ewe lambs and aged ewes treated with FSH and subjected to laparoscopic oocyte collection.

Age group	Ear Tag #	No. of Follicles Observed	No. of Follicles Aspirated	No. of Oocytes Collected	Percentage of Oocytes Collected *
Lamb	114	18	4	2	50.0
Lamb	181	24	24	14	58.3
Lamb	20	16	16	7	43.8
Lamb	133	40	40	27	67.5
Lamb	15	37	31	15	48.4
Lamb	1455	27	22	7	31.8
Total	6	162	137	72	52.6
Mean±SEM/Ewe		27±4.0[a]	22.8±5.1	12±3.6	52.6±5.0
Aged	10	14	14	4	28.6
Aged	93	16	16	4	25.0
Aged	24	9	9	4	44.4
Aged	34	14	15	3	20.0
Aged	62	30	30	11	36.7
Aged	87	24	22	17	77.3
Total	6	107	106	43	40.6
Mean±SEM /Ewe		17.8±3.1[b]	17.7±3.0	7.2±2.3	40.6±8.5

*Percentage of oocytes collected = (no. of oocytes collected/no. of follicles aspirated) x 1000

[a, b] values (means±SEM) are different; $P<0.10$.

Ewe lambs had more ($P<0.10$) visible follicles present on their ovaries than aged ewes. However, the number of follicles aspirated, the number of oocytes collected, and the percentage of oocytes collected were similar ($P>0.10$) for both the age groups.

DISCUSSION

Genetic improvement and livestock propagation by the conventional means of breeding is a slow process. For small ruminants, laparoscopic oocyte collection is the technique of choice for its simplicity, minimal invasiveness, repeatability and efficiency. Oocytes collected can be subsequently utilized for the in vitro production (IVP) of embryos (Earl and Kotaras, 1997). In addition, the rates of development are similar or even greater when the oocytes are recovered from live donors compared with oocytes collected from ovaries of slaughtered animals (Galli et al., 2001).

In the present experiment the recovery rate was 53% and 41% for lambs and aged ewes, respectively; other workers have shown similar or even higher results. A similar recovery rate of 49-54% was reported by Tervit et al.(1992) for ewes treated with PMSG. Stangl et al. (1999) also obtained a similar recovery rate (51 to 62%) when oocytes were collected once or twice a week from the same animal. In addition, Stangl et al. (1999) reported a greater recovery rate for ewes stimulated with PMSG compared with non-stimulated ewes, and also reported that 65-70% of the cumulus oocyte complexes (COC) were suitable for IVP of embryos. A greater rate of oocyte recovery ranging from 80 to 89% was reported by Baldassarre et al. (1994) and Earl et al.(1995) using the laparoscopic technique. These data demonstrate that the laparoscopic technique for oocyte collection is efficient and may provide large numbers of oocytes for IVP systems.

Data concerning the ability of oocytes collected via laparoscopy to fertilise in vitro are very limited at present. Only Tervit et al. (1995) reported the birth of lambs from embryos produced in vitro following laparoscopic recovery of oocytes. This indicates that this technique may find practical application for IVP of embryos. However, this subject requires further study.

In the present experiment the ewe lambs exhibited more visible follicles at collection compared with aged ewes, after FSH administration. Although the mechanisms responsible for this difference are currently unclear, it may result from differences in nutrition, breed, age or ability to respond to exogenous FSH administration. This unexplained difference represents another topic for future studies.

In summary, the present experiment demonstrates that laparoscopy is a feasible method of obtaining oocytes from donor ewes. The ewes lambs used in this study exhibited a better response to exogenous FSH administration and it seems that they may provide more oocytes for IVP of embryos. Future studies should confirm these age differences in FSH response, and also evaluate the quality of oocytes collected by laparoscopy by using in vitro fertilization procedures followed by embryo transfer.

ACKNOWLEDGMENTS

Supported in part by Hatch Project ND 01705 and ND SBARE.

LITERATURE CITED

Baldassarre H, de Matos DG, Furnus CC, Castro TE, Fischer EIC. Technique for efficient recovery of sheep oocytes by laparoscopic folliculocentesis. Animal Reprod Sci 1994; 35:145-150.

Earl CR, Irvine BJ, Kelly JM, Rowe JP, Armstrong DT. Development techniques for the production of viable embryos from 8 to 9 week old lambs. Proc Aust Soc Anim Prod 1994; 20:428 abstr.

Earl CR, Irvine BJ, Kelly JM, Rowe JP, Armstrong DT. Ovarian stimulation protocols for the oocyte collection and in vitro production of viable embryos from six to seven-week-old lambs. Theriogenology 1995; 43: 203 abstr.

Earl CR, Kotaras PJ. Oocyte retrieval and in vitro embryo production in sheep and cattle: current status and future perspectives. Aust J Agric Res 1997; 48: 125-135.

Galli C, Crotti G, Notari C, Turini P, Duchi R, Lazzari G. Embryo production by ovum pick up from live donors. Theriogenology 2001; 55:1341-1357.

Cognie Y. State of the Art in sheep-goat embryo transfer. Theriogenology 1999; 51: 105-116.

Gordon I. Controlled reproduction in sheep and goats. CAB International 1997.

Gourley, D.D., and Riese, R.M. 1990. Laparoscopic artificial insemination in sheep. Vet. Clin. NA: Food Anim. Prac. 6:615-633.

Guler A, Poulin N, Mermillod P, Terqui M, Cognie Y. Effects of growth factors, EGF and IGF-1, and estradiol on in vitro maturation of sheep oocytes. Theriogenology 2000; 54:209-218.

Naitana S, Loi P, Leda S, Cappai P. Embryo transfer and new technology in sheep production. In: A. Lauria and F. Gandolfi (editors) Embryonic development and manipulation in animal production: Trends in research and applications. Portland Press, London 1992; pp.183-194.

SAS User's Guide, Statistics, 5th Edn., 1985.Statistical Analysis Inst., Cary, NC.

Stenbak TK, Redmer DA, Berginski HR, Erickson AS, Navanukraw C, Toutges MJ, Bilski JJ, Kirsch JD, Kraft KC, Reynolds LP, Grazul-Bilska AT. Effects of follicle stimulating hormone (FSH) on follicular development, oocyte retrieval, and in vitro fertilization (IVF) in ewes during breeding season and seasonal anestrous. Theriogenology 2001; 56: 51-64.

Stangl M, Kuhholzer B, Besenfelder U, Brem G. Repeated endoscopic ovum pick-up in sheep. Theriogenology 1999; 52: 709-716.

Snyder DA, Dukelow R. Laparoscopic studies of ovulation, pregnancy and follicle aspiration in sheep. Theriogenology 1974; 2:143-148.

Tervit HR, Smith JF, McGowan LT, Wells RW, Parr J. Laparoscopic recovery of oocytes from sheep. Proc Aust Soc Reprod Biol 1992; 24:26 abstr.

Tervit HR, Smith JF, McGowan LT, Pugh PA. Birth of lambs from embryos produced in vitro following laparoscopic recovery of follicular oocytes. Proc Aust Soc Reprod Biol 1995; 27:68.

Pregnancy rates after transfer of *in vitro* produced (IVP) embryos: effects of epidermal growth factor (EGF)

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INTRODUCTION

The first sheep embryo transfer was reported about 70 years ago (Warwick et al., 1934). However, most of the embryo transfer technology has been developed within the last 20 years (Ishwar and Memon, 1996). The advantages of embryo transfer procedures are: recovering a large number of progeny from genetically superior females, easier introduction of exotic breeds, preserving endangered species, the opportunity for progeny testing females, minimizing the risk of introducing exotic diseases, minimizing cost and eliminating transportation stress in animals, obtaining twins and multiples from each pregnancy, utilizing genetically inferior females as foster mothers for embryos, and increasing the rates of genetic improvement (Ishwar and Memon, 1996; Earl and Kotaras, 1997; Gordon, 1997).

One of the technologies that increases the efficiency of embryo transfer is the in vitro production (IVP) of embryos. This procedure permits the production of a large number of offspring from living or slaughtered animals (Gordon, 1997; Loi et al., 1998; Guler et al., 2000). However, *in vitro* maturation (IVM), *in vitro* fertilization (IVF) and *in vitro* culture (IVC) results in a reduced percentage of transferable embryos compared to *in vivo* conditions (Van Wagtendonk-de Leeuw et al., 1998). Therefore, numerous experiments have been performed to improve embryo IVP systems.

Numerous supplements including gonadotropins, steroid hormones, growth hormone and several growth factors including EGF have been added to maturation, fertilization and/or culture media in order to improve the efficiency of IVP systems (Watson et al., 1994; Longergan et al., 1996; Abeydeera et al., 2000; Guler et al., 2000). Epidermal growth factor has been demonstrated to enhance oocyte maturation and blastocyst formation in several species (Coskun et al., 1991; Downs, 1989; Kim et al., 1999; Guler et al., 2000; Grazul-Bilska et al., 2001). It has been indicated that EGF can also have an effect on implantation (Kim et al., 1999).

The aim of this study was to evaluate the pregnancy rates after transfer of *in vitro* embryos produced in the presence or absence of EGF during *in vitro* maturation.

MATERIALS AND METHODS

Ewes of mixed breeds (n = 15) were injected twice daily (morning and evening) with FSH-P (Sioux Biochemical, Sioux Center, IA; Jablonka-Shariff et al., 1994; Stenbak et al., 2001) on days 13 (5 units/injection, day 0 = estrus) and 14 (4 units/injection) of the estrous cycle. On the morning of day 15, ovariectomy was performed (Reynolds et al., 1998). The number of visible

follicles on each ovary was counted. Oocytes were isolated by opening each visible follicle using a no. 15 scalpel blade and flushing it with oocyte collection medium two to three times using a Pasteur pipette in a petri dish (Watson et al., 1994; Stenbak et al, 2001; Grazul-Bilska et al., 2001. By using a stereoscope, oocytes were recovered from each dish and transferred to a petri dish containing fresh collection medium without heparin. Oocytes were then evaluated and categorized as healthy or atretic based on morphology according to Thompson et al. (1995). All oocytes were washed three times in maturation medium (TCM-199 containing 10 % FBS, ovine FSH [oFSH-RP-1; NIAMDD-NIH, Bethesda, MD], ovine LH [oLH-26; NIADDK-NIH], estradiol [Sigma], glutamine [Sigma], sodium pyruvate [Sigma], and penicillin/streptomycin; Stenbak et al, 2001). Before use, maturation medium was stabilized by incubating it overnight under oil at 39°C in 5% CO_2 and 95% air. For each ewe, half of the oocytes were incubated in maturation medium without EGF (control group), and the other half were incubated in the same medium containing 10 ng/ml EGF (Sigma; EGF group). Dose of EGF was chosen on the basis of previously published data (Longergan et al., 1996; Abeydeera et al., 2000; Guler et al 2000; Grazul-Bilska et al., 2001).

The oocytes were matured for 24 h at 39°C in 5% CO_2 and 95% air followed by cumulus cells removal by using a 1% hyaluronidase (Type I-S; Sigma) treatment. The oocytes were again evaluated for health based on morphology (Thompson et al., 1995). Oocytes classified as healthy were used for IVF. The oocytes were transferred to stabilized fertilization medium, consisting of synthetic oviductal fluid (SOF; Tervit et al., 1992) and 2% heat inactivated sheep serum collected from sheep on day 0-1 of the estrous cycle (O'Brien et al., 1997; Brown and Radziewic, 1998; Wang et al., 1998).

Frozen semen, which was pooled from 4 Hampshire rams, was thawed and viable sperm were separated using the swim up technique (Yovich, 1995; Stenbak et al, 2001). In this procedure, healthy and viable sperm from a semen fraction swim into the medium (modified sperm washing medium: Irvine Scientific, Santa Ana, CA), which lies on top of the thawed semen pool. This media containing the motile sperm was then centrifuged, the sperm were counted and 0.5-1.0x10^6 sperm/ml were added to the oocytes (up to 20 oocytes/500 µl/well). The oocytes were incubated with the sperm in fertilization media for 18 h at 39°C, 5% O_2, 5% CO_2 and 90% N_2. The embryos were then washed three times with culture medium without glucose (SOF supplemented with BSA, glutamine, MEM amino acids, BME amino acids [Sigma] and penicillin/ streptomycin; Catt et al., 1997; Stenbak et al, 2001), and cultured in the same medium for 24 h at 39°C, 5% O_2, 5% CO_2 and 90% N_2. The dishes were then evaluated to determine the number of fertilized oocytes. The fertilized oocytes were washed three times in culture medium containing glucose (Stenbak et al., 2001). After 48 hours, the developmental stage of the zygotes was evaluated and embryos in the stage of 16 or more cells were randomly selected for transfer on day 5 (day1 = day of fertilization).

Thirty-nine ewes of mixed breeds were selected to receive embryos on day 5 of the estrous cycle. The estrous cycles of the recipient ewes had been synchronized so that their expected day of ovulation coincided with the day of IVF. Synchronization consisted of a single i.m. injection of $PGF_2\alpha$ (Estrumate, Schering-Plough Animal Health Corp., Union, NJ. 1 cc, 0.5 doses) on day 8-12 of the estrous cycle.

For embryo transfer, recipient ewes were anesthetized as described by Gourley and Riese (1998). The abdominal cavity was inflated with CO_2 and a 1.5 mm diameter laparoscope was inserted through a 2 mm trocar approximately 5 cm anterior to the udder, and approximately 2 cm lateral to the midline. The ovary containing the functional corpus luteum (CL) was identified. A 5 cm incision was then made through the skin and body wall adjacent to the laparoscope and approximately 2-5 cm lateral to the midline. A Babcock forceps was inserted through this incision and the anterior portion (approximately 4-5 cm) of uterine horn ipsilateral to the ovary with the CL was clamped exteriorized. A Sovereign® Tom Cat catheter (Sherwood Medical, St Louis, MO, USA) containing two or three embryos in about 3 μL of culture medium was inserted approximately 2 to 4 cm into the tip of the uterine lumen through a small incision and the embryos were injected. The uterine horn was then replaced into the abdomen and the abdominal incision was sutured.

Before surgery, the recipients were on a pelleted diet as follows (% of diet dry matter): dehydrated beet pulp, 48.5%; dehydrated alfalfa, 24.3%; corn, 24.3%; soybean meal, 3.0%. This was fed at a level (dry matter intake) of 862 g/d, which worked out on an as-fed basis to about 958 g/d assuming 90% dry matter. Immediately after surgery (day 0), the ewes received 0.5 kg of chopped grass hay plus 1/3 the normal amount (0.32 kg) of the pelleted diet. On the day after surgery (day 1), ewes were fed a 0.5 kg of chopped grass hay plus 2/3 the normal amount (0.63 kg) of the pelleted diet. On the second day after surgery (day 2), ewes received no grass hay but they received the full amount (1 kg) of the pelleted diet. On the third day after surgery (day 3), ewes were put back on the normal feeding schedule. To verify pregnancy, the recipient ewes were placed with vasectomized rams beginning on day 6 after embryo transfer. In addition, the presence of fetuses was determined by ultrasonography (Classic Ultrasound Equipment Ltd, Tequesta, FL) on day 45 or after of embryo transfer.

All data are reported as means ± standard error (SEM). Data were analyzed by using the general linear models (GLM) procedure of the Statistical Analysis System (SAS, 1985) with the main effect of EGF. In addition, data for the percentage of oocytes cleaved and the percentage of pregnancy were analyzed by Chi-square (Spiegel, 1961).

RESULTS

The number of follicles was 22.9 ± 1.4/ewe, number of recovered oocytes was 23.9 ± 1.7/ewe, number of healthy oocytes was 21.6 ± 1.6/ewe, number of atretic oocytes was 2.3 ± 0.7/ewe and percentage of healthy oocytes was 90.5 ± 1.9 %/ewe. Total number of oocytes used for IVF was 370; 166 oocytes were matured with EGF, and 204 oocytes were matured without EGF.

EGF affected the morphology of the cumulus oocyte complex (COC). After maturation, cumulus cells were more expanded in cultures with EGF than without EGF. Table 1 presents the number of oocytes used for IVF and the number and percentage of fertilized oocytes after maturation with or without EGF.

Table 1. The number of oocytes used for IVF and number and percentage of fertilized oocytes after maturation with or without EGF.

Treatment Group	No. of oocytes used for IVF		No. of fertilized oocytes (%)		No. of oocytes not fertilized (%)	
	Total	Per ewe	Total	Per ewe	Total	Per ewe
Control	204	12.8 ± 1	120* (59%*)	7.5 ± 0.8	84 (35%)	5.3 ± 0.8
EGF	166	11.9 ± 1.1	129 (78%)	9.2 ± 0.9	37 (25%)	2.6 ± 0.8

* Values differ within a column ($P<0.05$)

The fertilization rate was greater ($P<0.05$) for oocytes matured with EGF (78%) than without EGF (59%).

Table 2 shows the effects of EGF on the rate of pregnancy.

Table 2. Pregnancy rates after transfer of embryos produced by IVF after maturation with or without EGF.

Treatment Group	No. of recipient ewes	No. of pregnancies (%)
Control	18	7 (39)
EGF	21	11 (52)

Pregnancy rates were similar ($P > 0.1$) for both treatment groups.

DISCUSSION

Although it has been reported that epidermal growth factor plays an important role in the control of cell proliferation and differentiation in general, EGF involvement in the regulation of early embryonic development and implantation is poorly understood.

The data of the present experiment demonstrated that presence of EGF in maturation medium resulted in a 19% increase (78% vs. 59%) in the rate of fertilization but did not affect pregnancy rates after transfer of embryos produced *in vitro*. Previous reports demonstrated that EGF did not affect the rates of fertilization but increased the rate of blastocyst formation in sheep (Guler et al., 2000; Grazul-Bilska et al., 2001). In the present experiment, the rate of blastocyst formation was not determined since embryos were transferred too early (on day 5 after fertilization) for blastocyst to be formed. The discrepancies between studies in terms of the effects of EGF on the rates of fertilization are likely due to culture conditions, breed and condition of ewes. However, these reports indicate that EGF in maturation medium is desirable to obtain a large number of *in vitro* produced embryos for further transfer.

Similar rates of fertilization of ovine oocytes, which were matured and fertilized in medium under various conditions but without EGF, were reported by others (60 % by Wang et al., 1998; 70 % by Stenbak et al., 2001; 72 % by Ledda et al., 1997; 82 % by O'Brien et al., 1997; 74 % by

Watson et al., 1994). This suggests that some specific culture conditions may provide a similar environment for high fertilization rates and further embryonic development as in our present experiment.

The pregnancy rates were similar for both groups (overall 46 %). Pregnancy rates reported by others varied from 29 to 65 % (Rexroad and Powell, 1990; Slavik et al., 1992; Thompson et al., 1995; Brown et al., 1998; Ptak et al., 1999). These differences may be due to the *in vitro* culture conditions, stage of development of transferred embryos, hormonal treatment, breed and location. However, our experiment demonstrated that transfer of embryos on day 5 is very efficient. Transfer of embryos on day 5 allows for shortening the time of embryo culture and the application of a laparoscopical technique for transferring embryos to the recipient ewes.

In summary, these data demonstrate that the presence of EGF in maturation medium increases the rate of fertilization but does not affect the rate of pregnancy in ewes during the normal breeding season. In addition, transfer of embryos on day 5 resulted in a relatively high and satisfactory rate of pregnancy.

ACKNOWLEDGMENTS

Supported in part by Hatch Project ND 01705 and ND SBARE. The authors thank Dr. Joel Caton for expert technical assistance with feed method after embryo transfer, and Wes Limesand for technical assistance in animal care.

LITERATURE CITED

Abeydeera, L.R., Wang, W.H., Cantley, T.C., Rieke, A., Murphy, C.N., Prather, R.S. and Day, B.N. 2000. Development and viability of pig oocytes matured in protein-free medium containing epidermal growth factor. Theriogenology 54: 787-797.

Brown, B.W., and Radziewic, T. 1998. Production of sheep embryos *in vitro* and development of progeny following single and twin embryo transfer. Theriogenology 49: 1525-1536.

Catt, S.L., O'Brien, J.K., Maxwell, W.M.C., and Evans, G. 1997. Effects of rate of development of *in vitro*-produced ovine embryos on sex ratio and *in vivo* survival after embryo transfer. Theriogenology 48: 1369-1378.

Coskun, S., Sanbuissho, A., Lin, Y.C., and Pikihisa, Y. 1991. Fertilization and subsequent development ability of bovine oocytes matured in medium containing epidermal growth factor (EGF). Theriogenology 36: 485-494.

Downs, S.M. 1989. Specificity of epidermal growth factor (EGF) action on maturation of the murine oocyte and cumulus oophorus *in vitro*. Biol Reprod 41: 371-379.

Earl, C.R., and Kotaras, P.J. 1997. Oocyte retrieval and *in vitro* embryo production in sheep and cattle: current status and future perspectives. Aus J Agric Res 48: 125-135.

Gordon, I. 1997. Controlled Reproduction in Sheep and Goats. CAB International.

Gourley, D.D., and Riese, R.M. 1990. Laparoscopic artificial insemination in sheep. Vet. Clin. NA: Food Anim. Prac. 6:615-633.

Grazul-Bilska, A.T., Choi, J.T., Bilski, J.J., Weigl, R.M, Kraft, K.C., Reynolds, L.P., and Redmer, D.A. 2001. Effects of epidermal growth factor (EGF) on oocyte maturation, *in vitro* fertilization (IVF) and blastocyst formation in ewes treated with follicle stimulation hormone (FSH). Western Dakota Sheep Day, Hettinger, ND, Report 42: 5-11.

Guler, A., Poulin, N., Mermillod, P., Terqui, M., and Cognie, Y. 2000. Effect of growth factors, EGF and IGF-1, and estradiol on *in vitro* maturation of sheep oocytes. Theriogenology 54: 209-218.

Ishwar, A.K., and Memon, M.A. 1996. Embryo transfer in sheep and goats: a review. Small Ruminant Res 19: 35-43.

Jablonka-Shariff, A., Fricke, P.M., Grazul-Bilska, A.T., Reynolds, L.P., and Redmer, D.A. 1994. Size, number, cellular proliferation, and atresia of gonadotropin- induced follicles in ewes. Biol Reprod 51: 531-540.

Kim, C.H., Chae, H.D., Cheon, Y.P., Kang, B.M., Chang, Y.S., and Mok, J.E. 1999. The effect of EGF on the preimplantation development, implantation and its receptor expression in mouse embryos. J Obstet Gynaecol Res 25: 87-93.

Ledda, S., Bogliolo, L., Calvia, P., Leoni, G., and Naitana, S. 1997. Meiotic progression and developmental competence of oocytes collected from juvenile and adult ewes. J Reprod Fertil 109; 73-78.

Loi, P., Ptak, G., Dattena, M., Ledda, S., Naitana, S., and Cappai, P. 1998. Embryo transfer and related technologies in sheep reproduction. Reprod Nutr Dev 38: 615-628.

Lonergan, P., Carolan, C., Langendonckt, A.V., Khatir, H., and Merimillod, P. 1996. Role of epidermal growth factor in bovine oocyte maturation and preimplantation embryo development *in vitro.* Biol Reprod 54: 1420-1429.

O'Brien, J.K., Catt, S.L., Ireland, K.A., Maxwell, W.M.C., and Evans, G. 1997. In vitro and *in vivo* developmental capacity of oocytes from prepubertal and adult sheep. Theriogenology 47: 1433-1443.

Ptak, G., Dattena, M., Loi, P., Tischner, M., and Cappai, P. 1999. Ovum pick-up in sheep: efficiency of *in vitro* embryo production, vitrification and birth of offspring. Theriogenology 52: 1105-1114.

Rexroad, C.E. Jr., and Powell, A.M. 1990. Effect of serum-free co-culture and synchrony of recipients on development of cultured sheep embryos to fetuses. J Anim Sci 69: 2066-2072

Reynolds, L.P., Kirsch, J.D., Kraft, K.C., Knutson, D.L., McClaflin, W.J. and Redmer, D.A. 1998. Time-course of the uterine response to estradiol-17 β in ovariectomized ewes: Uterine growth and microvascular development. Biol Reprod 59: 606-612.

SAS User's Guide, Statistics, 5th Edn., 1985. Statistical Analysis System Inst., Cary, NC.

Slavik, T., Fulka, J., and Goll, I. 1992. Pregnancy rate after the transfer of sheep embryos originated from randomly chosen oocytes matured and fertilized *in vitro*. Theriogenology 38: 749-756.

Spiegel, M.R. 1961. Theory and problems of statistics. Schaum's outline series, McGraw-Hill book co. New York, NY.

Stenbak, T.K., Erickson, A.S., Berginski, H., Toutges, M.J., Redmer, D.A., Reynolds, L.P., and Grazul-Bilska, A.T. 2001. Effects of follicle stimulating hormone (FSH) on follicular development, oocyte retrieval, and *in vitro* fertilization (IVF) in ewes during breeding season and seasonal anestrus. Theriogenology 56: 51-64.

Tervit, H.R., Whittingham, D.G., and Rowson, L.E.A. 1992. Successful culture *in vitro* of sheep and cattle ova. J Reprod Fertil 30: 493-497.

Thompson, J.G., Gardner, D.K., Pugh, P.A., McMillan, W.H., and Tervit, H.R., 1995. Lamb birth weight is affected by culture system utilized during *in vitro* Pre-elongation development of ovine embryos. Biol Reprod 53: 1385-1391.

Van Wagtendonk-de Leeuw, A.M., Aerts, B.J.G., and Den Daas, J.H.G. 1998. Abnormal offspring following *in vitro* production of bovine preimplantation embryos: a field study. Theriogenology 49: 883-894.

Watson, A.J., Watson, P.H., Warnes, D., Walker, S.K., Armstrong, D.T., and Seamark, R.F. 1994. Preimplantation development of *in vitro*-matured and *in vitro*-fertilized ovine zygotes: comparison between co-culture on oviduct epithelial cell monolayers and culture under low oxygen atmosphere. Biol Reprod 50: 725-733.

Wang, S., Liu, Y., Holyoak, G.R., Evans, R.C., and Bunch, T.D. 1998. A protocol for *in vitro* maturation and fertilization of sheep oocytes. Small Rum Res 29: 83-88.

Warwick, B.L., Berry, R.O., and Horlacher, W.R. 1934. Results of mating rams to angora female goats. Proc. Ann. Meeting Am. Soc. Anim. Prod., pp. 225-335.

Yovich, J.L. 1995. Gametes-The spermatozoon. Grudzinskas JG (ed.) & Yovich JL (ed) Cambridge University Press.

Effects of Pregnant Mare's Serum Gonadotropin on the Incidence of Estrus and Pregnancy Rates in Ewes Synchronized with Controlled Internal Drug Release Devices or Sponges and Subjected to Laparoscopic Artificial Insemination During the Breeding Season

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INTRODUCTION

Artificial insemination (AI) is a useful technique for improving reproductive performance in ewes as well as providing a means to introduce new genetics. Many different techniques have been used for AI. However, direct uterine insemination with the aid of a laparoscope has become the "industry standard" for AI in ewes, because of the relatively high conception rates compared to other techniques (Gourley and Riese, 1990). Laparoscopic AI (LAI) requires the use of estrus synchronization and timed insemination techniques, for LAI otherwise would be virtually impossible from a labor stand-point.

Many methods have been developed for synchronization of estrus in sheep (Maxwell, 1984; Gordon, 1997), but the most successful have been based on suppression of the estruos cycle with synthetic progestagens (Robinson, 1965; Maxwell and Barnes, 1986; Gourley and Riese, 1990; Gordon, 1997; Redmer et al., 2001). Perhaps the most widely used technique is that reviewed by Gourley and Riese (1990) and Redmer et al. (2000, 2001). This technique uses a synthetic progestin implant (Synchro-Mate B [SMB]) to synchronize estrus along with pregnant mare's serum-gonoadotropin (PMSG) to stimulate ovarian activity. However, the recent unavailability of SMB has forced the investigation of other progestagen-based therapies used to synchronize estrus by our laboratory.

A study by Maxwell and Barnes (1986), presented a comparison between the CIDR device and the progestin sponge in combination with PMSG for the induction and synchronization of estrus for AI. The authors revealed that synchronization of estrus and fertility was similar with CIDR devices and sponges. However, the effect of PMSG on the incidence of estrus and rates of pregnancy were left undetermined.

Therefore, this study was conducted to determine the effects of CIDR devices or progestin sponges and an injection of PMSG on synchronization of estrus and pregnancy rates following LAI during the breeding season (September, 2001).

MATERIALS AND METHODS

Purebred Hampshire, Columbia, and Suffolk ewes (n=8-14/breed) were randomly assigned to one of four treatments (n=8-10/group; each consisting of all three breeds) in a 2 x 2 factorial design (CIDR or sponge and -/+ PMSG). At CIDR device (contains 0.3 grams progesterone; InterAg, Hamilton, New Zealand) or progestin-impregnated sponge (Chronogest®, contains 30 mg chronolone; Intervet, Cambridge, England) removal, ewes received one intramuscular (i.m.)

injection of PMSG (Folligon, Intervet, Whitby, Ontario; 400 IU) or a vehicle (V). Vasectomized rams with markers were then penned with the ewes and estrus activity was recorded. Ewes were subjected to LAI 54-56 h after CIDR or sponge removal. Intact rams with markers were turned in with the ewes 10 days after LAI and rebreeding was recorded. Ewes were evaluated for pregnancy 35-40 days after LAI by real time ultrasonography and only pregnancies resulting from LAI were recorded.

RESULTS

Data regarding synchronization of estrus, pregnancy rates to LAI, and estrous return rates are presented in Table 1. No differences were observed among treatments (P>0.10; chi-square test) for any of the variables measured.

Table 1. Percentages in estrus, pregnant, and rebred for ewes synchronized with CIDR devices or sponges then subjected to laparoscopic artificial insemination following pregnant mare's serum gonadotropin treatment during the breeding season*

Treatment	n	Ewes in Synchronized Estrus† (%)	Pregnancy Rate to LAI† (%)	Ewes Rebred† (%)
CIDR/V	9	100	33.3	55.6
CIDR/PMSG	10	90.0	60.0	20.0
Sponge/V	8	75.0	50	62.5
Sponge/PMSG	9	100	66.7	33.3
Total	36	83.3	52.7	41.7

*Estrus refers to the estrus after CIDR or sponge removal and PMSG treatment; pregnant refers to pregnancy diagnosed by ultrasonography at 35-40 days after LAI, and pregnant to LAI; rebred refers to breeding mark at next estrus.

†No differences were observed among treatments (P>0.10) for Ewes in Synchronized Estrus, Pregnancy Rate to LAI, or Ewes Rebred by Chi-square test.

DISCUSSION

A majority of the ewes (83.3%) came into estrus between 24 and 48 h following sponge or CIDR device removal and ewes in a synchronized estrus was similar across treatments. In a study by Maxwell and Barnes (1986), the majority of ewes came into estrus between 24 and 48 h after sponge or CIDR device removal, with 96% of all ewes in estrus at 48 h. They reported no difference between the two devices in time of detection of estrus. Contradictorily, Welch et al. (1984) and Harvey et al. (1984) claimed CIDR devices provided a better synchrony of estrus than sponges.

Treatment with CIDR devices and sponges provides a similar pregnancy rate to timed insemination by LAI in seasonally estrous ewes. Maxwell and Barnes (1986) reported similar

fertility from natural mating and LAI following treatment with CIDRs and sponges combined with PMSG during seasonal estrus. Additionally, Crosby et al. (1983) reported similar fertility following mating of ewes synchronized with sponges and CIDRs and treated with PMSG during late anestrus.

Pregnant mare's serum gonadotropin has been used widely used in estrus synchronization programs in sheep (Maxwell and Barnes, 1986; Gourley and Reise, 1990; Redmer et al., 2000, 2001; Barrett et al., 2001). Typically, PMSG is used to stimulate ovarian activity during seasonal anestrous, and is usually used following estrous synchronization (Barrett et al., 2001). However, some potential short term and/or long term risk occurs with PMSG. Production of antibodies against PMSG may result in ovarian dysfunction, and over stimulation of follicular growth can result in production of multiple births in excess of two lambs (Redmer et al., 2000). Our objective was to determine if PMSG is necessary for the stimulation and synchronization of estrus during the normal breeding season. The results from the current study and previous studies (Redmer et al., 2000, 2001) have shown that PMSG has no significant effect on the percentage of ewes expressing a synchronized estrus or on the percentage of ewes conceiving to LAI.

In conclusion, results from the study herein indicate that incidence of estrus and fertility from LAI are similar following treatment with CIDR devices and sponges, and PMSG has shown to have no significant effect. It is important to note, however, that a larger scale study would be necessary to detect small but significant effects. Future studies regarding the optimization of procedures used to synchronize estrus in ewes both during and after the breeding season will provide improved pregnancy rates and overall success of these assisted reproductive techniques.

REFERENCES

Barrett, D.M.W., Bartlewski, P.M., and Rawlings, N.C. 2001. Ultrasound and endocrine evaluation of the ovarian response to a 12-day medoxyprogesterone sponge and single injection of pregnant mare's serum gonadotropin in ewes in seasonal anestrous. Biol. Reprod. 64 (Suppl. 1) p. 152 (Abstr. #117), 2001.

Crosby, T.F., Boland, M.P., and Gordon, I., 1983. Effect of progesterone/progestagen treatments on the induction of pregnancy in ewes during late anestrous. J. Anim. Sci. Suppl. 1, 471.

Gordon, I., Controlled Reproduction in Sheep and Goats, CAB International, 1997.

Gourley, D.D., and Riese, R.M. 1990. Laparoscopic artificial insemination in sheep. Vet. Clin. NA: Food Anim. Prac. 6:615-633.

Harvey, T.G., Johnson, D.L., Tervit, H.R. and Welch, R.A.S. 1984. Synchronization and artificial insemiation of ewes-techniques which ahve possible commercial application. Proc. New Zealand Soc. Anim. Prod. 44, 7-9.

Maxwell, W.M.C. 1984. Current problems and future potential of artificial insemination programs. In: Reproduction in Sheep (ed. D.R. Lindsay and D.T. Pearce), Australian Academy of Science and Australian Wool Corporation, Canberra, A.C.T., pp. 291-298.

Maxwell, W.M.C., Barnes, D.R. 1986. Induction of estrus in ewes using a controlled internal drug release device and PMSG. J. Agric. Sci., Cambridge. 106:201-203.

Redmer, D.A., Haugen, R.G., Stenbak, T.K., Arnold, D.R., Toutges, M.J., Berginski, H.R., Navanukraw, C., Limesand, W. Kirsch, J.D., Kraft, K.C., Bilski, J.J., Grazul-Bilska,

A.T., Gourley, D.D., Riese, R.L., and Reynolds, L.P. 2000. Effects of gonadotropin treatment on incidence of estrus and pregnancy rate in ewes synchronized with synchronized with Synchro-Mate-B (SMB) and subjected to laparoscopic artificial insemination (LAI) during the breeding season. Western Dakota Sheep Day. Report No. 41:93-96.

Redmer, D.A., Lopes, F., Choi, J.T., Arnold, D.R., Navanukraw, C., Limesand, W., Kraft, K.C., Bilski, J.J., Weigl, R., Haugen, R.G., Grazul-Bilska, A.T., Gourley, D.D., and Reynolds, L.P. 2001. Year 2: Effects of gonadotropin treatment on incidence of estrus and pregnancy rate in ewes synchronized with synchronized with Synchro-Mate-B (SMB) and subjected to laparoscopic artificial insemination (LAI) during the breeding season. Western Dakota Sheep Day. Report No. 42:1-4.

Robinson, T.J. 1965. Use of progestagen-impregnated sponges inserted intravaginally or subcutaneously for the control of the oestrous cycle in the sheep. Nature, 206, 39-41.

SAS. User's Guide, Statistics, 5th Edition., Statistical Analysis System Inst., Cary, NC. 1985.

Welch, R.A.S., Andrewes, W.D. Barnes, D.R., Bremner, K. and Harvey, T.G. 1984. CIDR dispensers for estrus and ovulation control in sheep. Proceedings of the 10th International Congress on Animal Reproduction and Artificial Insemination, Urbana, 1984, I, 354.

Effects of Melatonin and Controlled Internal Drug Release (CIDR) Device on Follicular Development and Oocyte Quality in the Anestrous Ewes Treated with Follicle Stimulating Hormone

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ABSTRACT

Administration of exogenous melatonin (MEL) and progesterone (P_4) in conjunction with follicle stimulating hormone (FSH) affects the number of developing follicles and oocyte quality in the anestrus ewe. Crossbred Rambioullet x Targhee Western range ewes (n=25) were randomly assigned to four treatment groups in a 2 x 2 factorial design [+/-MEL and +/- CIDR device; MEL/CIDR, CIDR, MEL, and Control (no treatment), respectively]. MEL/CIDR and MEL ewes (n=14) received an 18 mg Melovine® (melatonin) implant for 42 days before oocyte collection. MEL/CIDR and CIDR ewes (n=11) were vaginally implanted with CIDR (Type G) devices (intravaginal pessaries containing P_4) for five days before oocyte collection. Two days before oocyte collection all ewes received FSH injections twice daily. At slaughter, ovaries were removed, all visible follicles were counted, and oocytes were collected, matured and fertilized *in vitro*. The average number of follicles was greater ($P<0.08$) for MEL/CIDR ewes than Control ewes (37.3±5.5 and 22.6±5.5, respectively), but not different from MEL (31.3±5.5) and CIDR (25.8±5.5) ewes. Percentage of oocytes recovered from follicles was similar ($P>0.10$) for all treatments (overall 89.9±7.1%). Additionally, the rates of maturation of oocytes were similar ($P>0.10$) across treatments (overall 78.6±11.0%). Oocytes collected from CIDR treated ewes (CIDR and MEL/CIDR treatment groups), had lower ($P<0.02$) fertilization rates than ewes not treated with CIDR (MEL and Control; 10.3±2.0 and 10.1±2.0 vs. 18.5±2.0 and 20.0±2.0%, respectively). These data indicate that melatonin and P_4 increases the number of developing follicles, and although P_4 assists in the recruitment of more follicles, it decreases fertilization rates.

INTRODUCTION

The ewe is seasonally polyestrous and will stop cycling during late winter in response to the increasing photoperiod (Robinson et al., 1993; Bittman, 1984). Low fertilization rates during seasonal anestrous may be caused by an altered endocrine status when compared to the normal breeding season in the ewe (Stenbak et al., 2001). Numerous studies have focused on developing hormonal treatments to improve follicular development and induce estrus in ewes during seasonal anestrus (Robinson et al., 1991 and 1993; Gordon, 1997; Carlson, 2000; Knights et al., 2000, 2001). The main focus of these previous studies was to improve pregnancy rates and maximize reproductive performance in vivo. However, limited data are available concerning the effects of exogenous hormones, such as melatonin and progesterone, on oocyte quality for in vitro fertilization (IVF) during seasonal anestrus.

Many studies have been conducted during seasonal anestrus to evaluate the effectiveness of different melatonin treatments on reproductive performance in vivo. Melatonin treatment has been shown to be an effective method to induce estrous cycles, increase ovulation rates, and increase lambing rates during seasonal anestrous (Waller, 1988; Haresign, 1990 and 1992; Robionson 1991 and 1993; Bister, 1999; Carlson, 2000). It has also been demonstrated that melatonin affects oocyte development and support fertilization and early embryonic development following IVF in rats and mice (Fernandez, 1995; Ishizuka, 2000).

Another common method of inducing fertile estrus in the seasonal anestrous ewe is through the use of progesterone-based therapies (Robbinson et al., 1991; Jabbar et al., 1994; Knights et al., 2000, 2001). An improvement in fertility of ewes synchronized with higher doses of progesterone is due to an increase in sperm transport (Hawk, 1971), synchrony in the onset of estrus in relationship to the luteinizing hormone surge (Van Cleeff, 1998), and/or patterns of follicular development (Johnson, 1996). Knights et al. (2001) demonstrated that a 5-day treatment with progesterone, in combination with follicle stimulating hormone (FSH), stimulated a fertile estrus as effectively as a 12-day progesterone treatment with FSH. In addition, this resulted in prolificacy comparable to that observed during the normal breeding season. However, data concerning the effects of melatonin and/or progestagen treatment on quality of ovine oocytes are not available at present.

The aim of this study was to evaluate the effects of exogenous melatonin and progesterone on follicular development and oocyte quality in FSH-treated ewes. Oocyte quality was measured by the rate of maturation, fertilization, and morula and blastocyst formation following *in vitro* fertilization procedures.

MATERIALS AND METHODS

Animals and Experimental Design

Seasonally anestrous, crossbred Rambioullet/Targhee Western range ewes (n=25) were randomly assigned to four treatment groups (n=4-7/group) in a 2x2 factorial design [+/- melatonin (MEL) and +/-CIDR device]. Ewes received an 18 mg Melovine® (melatonin; Sanofi Sante Nutrition Animal, La Ballastiere, France) implant for 42 days before oocyte collection and were vaginally implanted with CIDR devices for five days before slaughter (day of oocyte collection). Two days before slaughter all ewes received FSH injections as described by Stenbak et al. (2001). This study was conducted during the period from March to May

Follicular Evaluation and Oocyte Collection

Ovaries were removed at slaughter and placed in phosphate buffer solution (PBS) containing penicillin/streptomycin (Gibco, Gaithesburg, MD) at 39° C. The number of visible follicles on each ovary were counted. Oocytes were collected using a no. 15 scalpel and a Pasteur pipette in a petri dish containing oocyte collection media (Stenbak et al., 2001). Each follicle was cut with a scalpel and washed/flushed two or three times. Oocytes were then evaluated based on morphology and categorized as healthy or atretic according to Thompson et al. (1995). All oocytes were washed three times before being transferred into maturation medium containing

epidermal growth factor (EGF; Choi et al., 2001; Grazul-Bilska et al., 2001; Stenbak et al., 2001) stabilized under mineral oil.

In Vitro Maturation

Oocytes were matured for 21-24 h at 39° C, 5% CO_2, and 95% air, and then oocytes were evaluated again for health based on morphology (Thompson et al., 1995). Only healthy-looking oocytes were used for IVF. The cumulus cells were removed by using 0.1% hyaluronidase (Type I-S; Sigma) treatment (Stenbak et al., 2001). Following cumulus cell removal oocytes were transferred to stabilized fertilization medium, consisting of synthetic oviductal fluid (SOF; O'Brien et al., 1997; Tervit et al., 1992; Walker et al., 1996; Wang et al., 1998; Stenbak et al., 2001) and 2% heat inactivated sheep serum collected on day 0 of the estrous cycle.

In Vitro Fertilization and Culture

Frozen semen, pooled from 4 Hampshire rams, was thawed and viable sperm were separated using the swim-up technique in modified sperm washing medium (Irvine Scientific, Santa Ana, CA; Yovich, 1995; Stenbak et al., 2001). The oocytes were fertilized with 0.5-1.0 x 10^6 viable sperm/mL (up to 20 oocytes/500 μL well). The oocytes were incubated with the sperm for 17 to 20 h at 39° C, 5% CO_2, 5% O_2, and 90 N_2. Then zygotes were cultured in SOF medium without glucose (Catt et al., 1997, Wang, 1998; Stenbak et al., 2001). The dishes were evaluated approximately 48 h after adding sperm to determine the rate of fertilization based on the number of cleaved oocytes.

Oocyte Staining to Determine Maturation Status

Oocytes that failed to fertilize were fixed in methanol and then stained with 0.1 μg/ml of 4,6-diamino-2-phenylindole (DAPI; Molecular Probes Inc., Eugene, OR, USA) in methanol for 15 minutes and mounted on slides (Jablonka-Shariff and Olson, 2000). The evaluation of nuclear status was done by epifluorescence microscopy (Gardner et al., 1997). Oocytes in the germinal vesicle stage, containing diplotene chromatin were considered to be immature. Mature oocytes demonstrated exclusion of the first polar body and therefore, were found to be in Metaphase II (Gaudet et al., 1997).

Statistical Analysis

Numbers of follicles and oocytes collected, and numbers and percentages of matured oocytes and cleaved zygotes were analyzed by using the general linear models procedure of the Statistical Analysis System (User's Guide, 1985). When the F-test was significant, differences between specific means were evaluated using the least square differences test (Kirk, 1982). Rates of oocyte maturation and fertilization were analyzed by using the Chi-Squared procedure of the Statistical Analysis System (User's Guide, 1985).

RESULTS

Figure 1 presents the average number of visible follicles on the ovaries of Control, CIDR, MEL, and MEL/CIDR ewes.

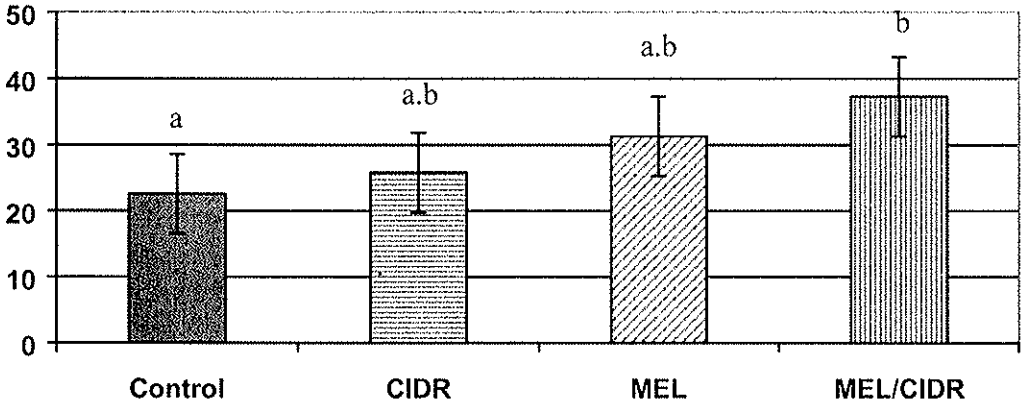


Figure 1. Average number of visible follicles for Control, CIDR, MEL, and MEL/CIDR ewes. [a,b] values are different; P<0.08. Data (mean ±SEM) are expressed per ewe.

The average number of follicles was greater (P<0.08) for MEL/CIDR ewes than Control ewes (37.3±5.5 and 22.6±5.5, respectively), but similar to MEL (31.3±5.5) and CIDR (25.8±5.5) ewes.

Table 1 presents the number and percentage of healthy oocytes (number of healthy oocytes/total number of oocytes recovered X 100) recovered from Control, CIDR, MEL, and MEL/CIDR ewes.

Table 1. Total number of oocytes and healthy oocytes recovered from Control, CIDR, MEL, and MEL/CIDR ewes.

		Recovered Oocytes		Healthy Oocytes		Healthy Oocytes (%)
Treatment	n	Per Ewe	Total	Per Ewe	Total	Total
Control	7	21.6±5.3	151	19.9±0.7	139	92.1
CIDR	4	21.8±7.1	87	21.5±7.4	86	98.9
MEL	7	27.3±5.3	191	26.3±0.7	184	96.3
MEL/CIDR	7	34.7±5.3	243	33.0±0.7	231	95.1
Overall	25	28.6±7.1	672	25.2±7.4	640	95.2

n=number of ewes

Total number of oocytes, healthy ooctyes and percentage of healthy oocytes from Control, CIDR, MEL, and MEL/CIDR ewes was similar (P>0.10) across treatments.

Table 2 presents the total number of oocytes analyzed for maturation status (fertilized oocytes and non-fertilized oocytes analyzed with DAPI staining) and the maturation rate (number of matured oocytes/total number of oocytes analyzed for maturation status X 100) of oocytes collected from Control, CIDR, MEL, and MEL/CIDR ewes.

Table 2. Total number of oocytes analyzed for maturation status and the rate of maturation (%) for oocytes collected from Control, CIDR, MEL, and MEL/CIDR ewes.

		Oocytes Analyzed for Maturation		Matured Oocytes		Maturation Rate (%)
Treatment	n	Per Ewe	Total	Per Ewe	Total	Total
Control	7	13.6±3.5	95	9.8±2.9	69	72.6
CIDR	4	13.8±4.2	55	11.1±3.6	44	80.0
MEL	7	16.3±3.5	114	13.8±2.9	97	85.1
MEL/CIDR	7	18.4±3.5	129	33.0±2.9	99	76.7
Overall	25	15.5±4.2	393	12.2±3.6	309	78.6

n=number of ewes

Maturation rates of oocytes were similar (P>0.10) across treatment groups, ranging from 72.6-85.1% (Table 2).

Table 3 presents the total number of healthy oocytes used for the IVF portion of this studay and their corresponding fertilization rates (number of fertilized oocytes/number of oocytes used for IVF X 100) in Control, CIDR, MEL, and MEL/CIDR treated ewes.

Table 3. Number of oocytes used for IVF and their corresponding fertilization rates (%) in Control, CIDR, MEL, and MEL/CIDR treated ewes.

		Oocytes for IVF		Oocytes Fertilized		Fertilization Rate (%)
Treatment	n	Per Ewe	Total	Per Ewe	Total	Total
Control	7	18.6±6.1	130	3.7±1.5	26	20.0[a]
CIDR	4	21.8±8.0	87	2.3±2.0	9	10.3[b]
MEL	7	25.4±6.1	178	4.7±1.5	33	18.5[a]
MEL/CIDR	7	34.0±6.1	238	3.4±1.5	24	10.1[b]
Overall	25	25.0±8.0	633	3.5±2.0	92	14.5

n=number of ewes

[a,b] values are different with in a column; P<0.02.

Oocytes collected from P_4 treated ewes (CIDR and MEL/CIDR treatment groups), had lower ($P<0.02$) fertilization rates than MEL and Control ewes (10.3±2.0 and 10.1±2.0 vs. 18.5±2.0 and 20.0±2.0, respectively).

DISCUSSION

The number of follicles and the number of oocytes and embryos obtained from animals must be optimized to maintain high efficiency of assisted reproductive technologies (ART). Numerous studies have focused on hormonal treatments to optimize follicular development in ewes during seasonal anestrus for successful embryo production and subsequent pregnancy rates (Gordon, 1997). As shown in this study and previously, administration of exogenous FSH (Gordon, 1997; Reynolds et al., 1998; Stenbak et al., 2001), melatonin (Rajkumar, 1989; Wigzell et al., 1986), and progestagens (Waller et al., 1988; Rajkumar et al., 1989; Wheaton et al., 1990; Safranski et al., 1992) have been shown to promote ovarian activity and succeeding follicular development in the seasonally anestrus ewe.

Follicle stimulating hormone (FSH) has been shown to promote a large number of follicles on each ovary when injected into ewes for two or more days at regular intervals during the breeding season and seasonal anestrus (Gordon, 1997; Reynolds et al., 1998; Stenbak et al., 2001). Additionally, several studies have examined the effects of exogenous melatonin and progestagen administration on ovarian activity and follicular development in vivo (Wheaton et al., 1990; Waller, 1988; Carlson, 2000). A major role of melatonin is to coordinate seasonal changes in reproductive activity (Hazlerigg, 2001). Melatonin has been shown to increase ovulation rate and litter size in the seasonally anestrus ewe (Rajkumar, 1989; Haresign, 1992). The use of progestagens have also been shown to promote ovarian activity by increasing the number of follicles and rate of ovulation during seasonal anestrous in the ewe (Waller et al., 1988; Rajkumar et al., 1989; Wheaton et al., 1990; Safranski et al., 1992; Leyva, 1998; Knights et al., 2001). Progestagens-treatment has been used in conjunction with high levels of FSH to promote the development of a large number of follicles during seasonal anestrus (Reynolds et al., 1998; Stenbak et al., 2001) and during the natural breeding season (Gordon, 1997; Stenbak et al., 2001).

In addition to hormonal treatment, the 'ram effect' has been shown to stimulate an earlier start of the breeding season in the ewe. Introduction of the ram among ewes during seasonal anestrus has been shown to be an effective method at inducing ovulation and subsequent estrus (Gordon, 1997; Faller and Berg, 2001). Additionally, the 'ram effect' has been reported to be an important and integral part of melatonin treatment strategies (Oldham and Martin, 1978). The principal role of melatonin in previous studies has been to advance the period of sensitivity to the 'ram effect' and then to use ram introduction to promote a greater degree of synchrony in mating (Haresign, 1992). The role of the 'ram effect' on oocyte quality during seasonal anestrus remains unclear and requires further research.

In agreement to previous in vivo studies, which used exogenous melatonin and progestagen administration during seasonal anestrus (Rajkumar, 1989; Haresign, 1992), the current study demonstrated that the greatest number of follicles was achieved by administration of a slow-release melatonin implant in combination with a progesterone-releasing device and FSH-

administration. Therefore, this hormonal treatment allows for the generation of a greater number of oocytes to be used for IVF procedures.

In numerous studies a fertile estrus was induced during the non-breeding season with the use of progestagens (Waller et al., 1988; Rajkumar et al., 1989; Wheaton et al., 1990; Safranski et al., 1992; Leyva, 1998; Knights et al., 2001). In a study by Knights et al. (2001), ewes treated with P_4 and low levels of FSH demonstrated an increase in the number of follicles and a greater portion of P_4 treated ewes lambed to the first service period when compared to non-P_4 treated controls. Treatment with P_4 for 5 days was shown to be as effective as for 12 d to induce a fertile estrus in FSH-treated anestrous ewes.

Previous studies have shown that exposure of oocytes to various hormones in vivo causes maturational changes that are necessary for proper development to occur (Cheng, 1985; Pugh et al., 1991; Armstrong et al., 1994; Assey et al., 1994; Fernandez et al., 1995; Ishizuka et al., 2000; Stenbak et al., 2001). Optimal levels of exogenous gonadotropins shoud be used to promote proper oocyte development. Depending on the regime of gonadotropin treatment, positive or negative effects on oocyte maturation and fertilization were observed (Evans and Armstrong, 1984; Pugh et al., 1991; Assey et al., 1994; Greve et al., 1995; Stenbak et al., 2001). In the current study, maturation rates were relatively high and similar across treatment groups, ranging from 72.6-85.1%. Therefore, exogenous melatonin and progesterone administration does not appear to affect maturational ability of oocytes during seasonal anestrus in FSH treated ewes.

In the present study, oocytes collected from P_4 treated ewes (CIDR and MEL/CIDR treatment groups) had lower fertilization rates than ewes that were not treated with P_4. In a study by Stenbak et al. (2001), similar rates of fertilization were achieved following a 14-day progestagen (SMB) and two-day FSH treatment. However, the fertilization rates following administration of SMB twice for 14 days appear to be greater than a five-day CIDR treatment (27%; Stenbak et al., 2001). Although the use of progesterone-releasing devices appeared to decrease fertilization rates in the current study, different results may be revealed if used twice (Stenbak et al., 2001). However, the mechanisms for promoting an increased rate of fertilization following a treatment with SMB twice for 14 days are not fully understood.

In contrast, Pugh et al. (1991) reported relatively high IVF rates (about 50-60%) for oocytes matured in the presence of granulosa cells for FSH-treated and non-treated ewes during the non-breeding season. These differences may be due to hormonal treatment, culture conditions, breed and location.

Data concerning the effects of melatonin on IVF rates in larger animals are not available. In the present experiment, we did not observe any melatonin effects on the rates of maturation or fertilization. However, for rats and mice, melatonin has been shown to affect oocyte development and sustain fertilization and early embryo development after IVF (Fernandez et al., 1995; Ishizuka et al., 2000).

During seasonal anestrus the rates of IVF (about 10-30%) are much lower than during the normal reproductive season (about 70-80%; Watson et al., 1994; Ledda et al., 1997; O'Brien et al., 1997; Choi et al., 2001; Grazul-Bilska et al., 2001; Stenbak et al., 2001). Therefore, additional studies

need to be conducted in order to provide more optimal hormonal treatments for seasonal anestrous sheep to mimic the hormonal environment of sheep during the breeding season.

In conclusion, administration of melatonin and CIDR devices in conjunction with FSH to the anestrus ewe increases the number of developing follicles. However, CIDR-treatment decreased the rates of IVF. These data indicate that further research involving the use of hormonal treatment is needed to improve in vitro fertilization techniques for the seasonally anestrus ewe. The role of melatonin in the oocyte maturation and fertilization processes remains unclear and requires additional studies.

ACKNOWLEDGEMENTS

The authors would like to thank Sanofi Sante Nutrition Animal, La Ballastiere, France for donating the subcutaneous, melatonin implants (18 mg of melatonin; Melovine®).

REFERENCES

Armstrong DT, Irvine BJ, Earl CR, McLean D, Seamark RF. 1994. Gonadotropin stimulation regimens for follicular aspiration and in vitro embryo production from calf oocytes. Theriogenology 42: 1227-1236.

Assey RJ, Hyttel P, Greve T, Purwantara B. 1994. Oocyte morphology in dominant and subordinate follicles. Mol Reprod Dev 37: 335-344.

Bister JL, Noeal B, Perrad B, Mandiki SN, Mbayahaga J, Paquay R. 1999. Control of ovarian follicles activity in the ewe. Dom Anim Endocrinol 17: 315-328.

Bittman EL. 1984. Melatonin and photoperiodic time measurement: evidence from rodents and ruminatns. In: The Pineal Gland, Ed. Russel J. Reiter. Raven Press, New York, pp. 155-192

Carlson CD. 2000. The effect of melatonin dosage and progesterone on reproduction in anestrous ewes. http://www.csuchico.edu/agr/tempDocs/ccarlson.html.

Catt SL, O'Brien JK, Maxwell WMC, Evans G. 1997. Effects of rate of development of in vitro-produced ovine embryos on sex ratio and in vivo survival after embryo transfer. Theriogenology. 48: 1369-1378.

Cheng, W.T.K. In vitro fertilization of farm animal oocytes. Ph.D. Thesis, University of Cambridge. 1985. pp. 133-154.

Choi JT, Redmer DA, Reynolds LP, Bilski JJ, Weigl RM, Grazul-Bilska AT. 2001. Effects of epidermal growth factor on embryonic development after in vitro fertilization of oocytes collected from FSH treated ewes. Bio Reprod. 64 (Suppl. 1) p. 306 (Abstr. #508).

Evans G, Armstrong DT. 1984. Reduction in fertilization rate in vitro of oocytes from immature rats induced to superovulate. J Reprod Fertil. 70: 131-135.

Fernandez B, Diaz E, Colomenro MD, Diaz B. 1995. Maternal pineal gland participates in prepubertal rats' ovarian oocyte development. Anat Rec 243: 461-465.

Gaudet G, Bezard J, Duchmap G, Gerard N, and Palmer E. 1997. Equine oocyte competence for nuclear and cytoplasmic in vitro maturation: effect of follicle size and hormonal environment. Bio Reprod 57: 232-245.

Gordon I, Controlled Reproduction in Sheep and Goats, CAB International, 1997.

Grazul-Bilska AT, Choi JT, Bilski JJ, Weigl R, Kraft KC, Kirsch JD, Reynolds LP, Redmer DA 2001. Effects of epidermal growth factor (EGF) on oocyte maturation, in vitro fertilization (IVF) and blastocyst formation in ewes treated with follicle stimulating hormone (FSH). Western Dakota Sheep Day. Report No. 42: 5-11.

Greve T, Callesen H, Hyttel P, Hoier R, Assey R. 1995. The effects of exogenous gonadotropins on oocyte and embryo quality in cattle. Theriogenology 43: 41-50.

Haresign W. 1992. Manipulation of reproduction in sheep. Reprod Fertil (Suppl) 45:127-139.

Hawk HW, Conley HH. 1971. Loss of spermatozoa from the reproductive tract of the ewe and intensification of sperm breakage by progestagen. Reprod Fertil 27: 339-347.

Hazlerigg DG. 2001. What is the role of melatonin in the anterior pituitary? Endocrinology 170: 493-501.

Ishizuka B, Kuribayashi Y, Murai K, Amemiya A, Itoh M. 2000. The effect of melatonin on in vitro fertilization and embryo development in mice. Pin Res 28: 48-51.

Jabbar G, Umberger SH, Lewis GS. 1994. Melengestrol acetate and norgestomet for the induction of synchornized estrus in seasonally anovular ewes. J Anim Sci 72: 3049-3054.

Jablonka-Shariff A, Olson LM, 2000. Nitric oxide is essential for optimal meiotic matruation of murine cumulus-oocyte complexes in vitro. Mod Reprod Dev 55: 412-21.

Johnson SK, Dailey RA, Inskeep EK., Lewis PE. 1996. Effect of peripheral concentrations of progesterone on follicular growth and fertility in ewes. Dom Anim Endocrinol 13: 69-79.

Kirk RE. Experimental Design: Procedures for the Behavior Sciences. Brooks/Cole Publishing Co. 1982.

Knights M, Hoehn T, Lewis PE, Inskeep EK. 2000. Effectiveness of intravaginal progesterone inserts and FSH to induce synchronized estrus and increase lambing rate in anestrous ewes. Abstract presented at the Midwestern Section ASAS and Midwest Branch ADSA 2000 Meeting, Des Moines, IA. J Anim Sci. 78 (Suppl 2) 216 Abstr.

Knights M, Maze TD, Bridges PJ, Lewis PE, Inskeep EK. 2001. Short-term treatment with a controlled internal drug releasing (CIDR) device and FSH to induce fertile estrus and increase prolificacy in anestrous ewes. Theriogenology 55: 1181-1191.

Ledda S, Bogliolo L, Calvia P, Leoni G, Naitana S. 1997. Meiotic progression and developmental competence of oocytes collected from juvenile and adult ewes. J Reprod Fertil 109: 73-78.

Leyva V, Buckrell BC, Walton JS. 1998. Follicular activity and ovulation regulated by exogenous progestagen and PMSG in anestrous ewes. Theriogenology 50:377-393.

Matthews CD, Guerin MV, Deed JR. 1993. Melatonin and photoperiodic time measurement: seasonal breeding in sheep. J Pineal Res 14: 105-116.

Malpaux B, Thiery JC, Chemineau P. 1999. Melatonin and the seasonal control of reproduction. Reprod Nutr Dev 39: 355-366.

O'Brien JK, Catt SL, Ireland KA, Maxwell WMC, Evans G. 1997. In vitro and in vivo developmental capacity of oocytes from prepubertal and adult sheep. Theriogenology 47: 1433-1443.

Oldham CM, Martin GB. 1978. Stimulation of seasonally anovular merino ewes by rams. II. Premature regression of ram induced corpora lutea. Anim Reprod Sci 3: 291-295.

Pugh PA, Fukui Y, Tervit HR, Thompson JG. 1991. Developmental ability of in vitro matured sheep oocytes collected during the nonbreeding season and fertilized in vitro with frozen ram semen. Theriogenology 36: 771-778.

Rajkumar RR, Argo CM, Rodway RG. 1989. Fertility of ewes given either melatonin or progestogen sponges. Vet Rec 124: 215-217.

Reynolds LP, Kirsch JD, Kraft KC, Grazul-Bilska AT, Redmer DA. 1998. Use of a synthetic progestogen in combination with a superovulatory treatment for induction of synchronized estrus in seasonally anovular ewes. Anim Sci 76 (Suppl.): 245 abstr.

Robinson JJ, Wigzell S, Aitken RP, Wallace JM, Ireland S, Robertson IS. 1991. Daily oral administration of melatonin from March onwards advances the breeding season by 4 months of ewes maintained under the ambient photoperiod at 57 degrees N. Anim Reprod Sci 27: 141-160.

Robinson JJ, Wallace JM, Aitken RP, McNeilly AS. 1993. Effect of chronic treatment with a GnRH agonist to suppress pulsatile LH secretion on the ability of exogenous melatonin to advance oestrous cyclicity in ewes. Reprod Fertil 99: 601-608.

Safranski TJ, Lamberson WR, Keisler FH. 1992. Use of melengestrol acetate and gonadotropins to induce fertile estrus in seasonally anestrous ewes. Anim Sci 70: 2935-2941.

SAS User's Guide, Statistics, 5th Edition., Statistical Analysis System Inst., Cary, NC. 1985.

Stellflug JN, Rodriguez F, LaVoie VA, Glimp HA. 1994. Influence of simulated phoroperiod alteration and induced estrus on reproductive performance of spring-born Columbia and targhee ewe lambs. Anim Sci 72: 29.

Stenbak TK, Redmer DA, Berginski HR, Erickson AS, Navanukraw C, Toutges MJ, Bilski JJ, Kirsch JD, Kraft KC, Reynolds LP Grazul-Bilska AT. 2001. Effects of follicle stimulating hormone (FSH) on follicular development, oocyte retrieval, and in vitro fertilization (IVF) in ewes during breeding season and seasonal anestrous. Theriogenology 56: 51-64.

Tervit HR, Whittingham DG, Rowson LEA. 1992. Successful culture in vitro of sheep and cattle ova. J Reprod Fertil 30: 493-497.

Thompson JG, Gardner DK, Pugh PA, McMillan WH, Tervit HR. 1995. Lamb birth weight is affected by culture system utilized during in vitro pre-elongation development of ovine embryos. Biol Reprod 53:1385-1391.

Van Cleeff J, Karsch FJ, Padmanabhan V. 1998. Characterization of the endocrine events during the periestrous period in sheep after estrous synchronization with controlled internal drug release (CIDR) device. Dom Anim Endrocrinol 15: 23-34.

Viguie C, Caraty A, Locatelli A, Malpaux B. 1995. Regulation of luteinizing hormone-releasing hormone (LHRH) secretion by melatonin in the ewe. 1. Simulataneous delayed increase in LHRH and luteinizing hormone pulsatile secretion. Biol Reprod 52: 1156-1161.

Walker SK, Hill JL, Keemann DO, Nancarrow CD. 1996. Development of ovine embryos in synthetic oviductal fluid containing amino acids at oviductal fluid concentrations. Biol Reprod 55: 703-708.

Waller SL, Hudgens RE, Diekman MA Moss GE. 1988. Effect of melatonin on induction of estrous cycles in anestrous ewes. J Anim Sci 66: 459-463.

Wang S, Liu Y, Holyoak GR, Evans RC, Bunch TD. 1998. A protocol for in vitro maturation and fertilization of sheep oocytes. Small Ruminant Res 29: 83-88.

Watson AJ, Watson PH, Warnes D, Walker SK, Armstrong DT, Seamark RF. 1994. Preimplantation development of in vitro-matured and in vitro-fertilized ovine zygotes: comparison between coculture on oviduct epithelial cell monolayers and culture under low oxygen atmosphere. Biol Reprod 50: 715-724.

Wheaton, J.E., Pohl, H.A., and Windels, H.F. 1990. Effects of melatonin and progesterone administered to ewes in spring and summer. J Anim Sci 68:923-930.
Yovich JL. Gametes-The Spermatozoon. Grudzinskas J.G. (ed.) & Yovich J.L. (ed.), Cambridge University Press. 1995.

SECTION II
GUEST SPEAKER SECTION

PRESENTED BY

DR. SUSAN KELLER
NORTH DAKOTA DEPARTMENT OF ANIMAL HEALTH
BISMARCK, NORTH DAKOTA

AT THE

43rd ANNUAL SHEEP DAY

HETTINGER RESEARCH EXTENSION CENTER
HETTINGER, NORTH DAKOTA

FEBRUARY 13, 2002

USDA
National Scrapie Eradication Program

Diane Sutton
Michael Gilsdorf
USDA Scrapie Program Coordinators

presented by
Susan Keller

1/23/02

What is Scrapie?

- Scrapie is a fatal, degenerative disease affecting the central nervous system of sheep and goats.
- Scrapie is classified as a TSE, as are BSE and CWD

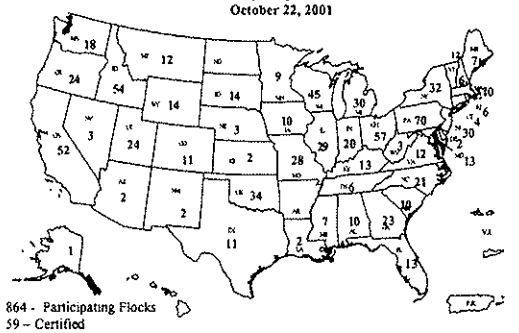
Clinical Signs

- Changes in behavior
- Scratching and rubbing
- Loss of coordination
- Lip smacking
- Gait abnormalities
- Weight loss
- Weakness, unable to rise

Current Situation

Scrapie Flock Certification Program

Participating Flocks on
October 22, 2001

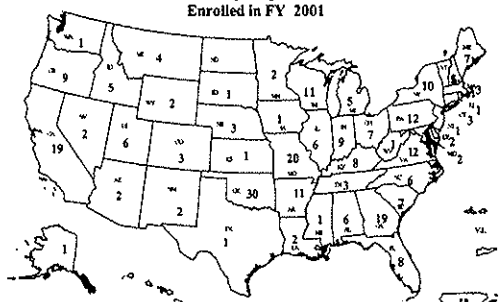


864 - Participating Flocks
59 – Certified
796 – Complete Monitored
9 – Selective Monitored

USDA

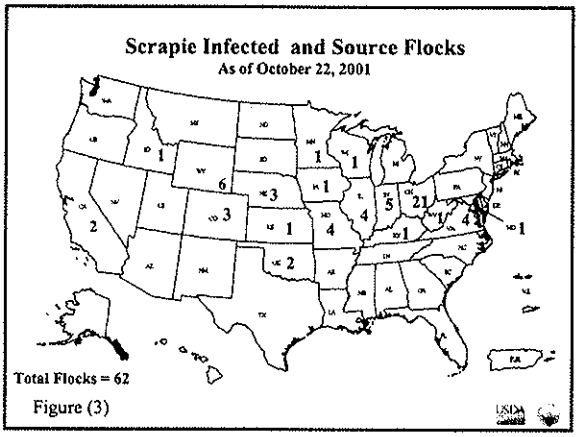
Scrapie Flock Certification Program

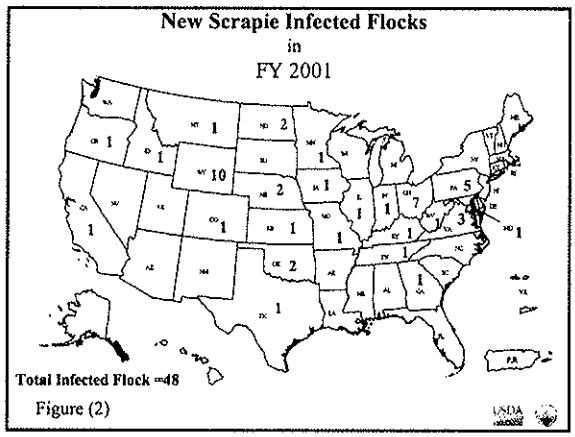
Participating Flocks
Enrolled in FY 2001

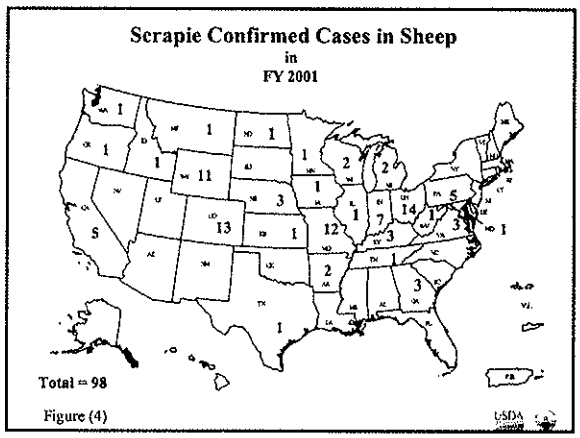


Total New Participating Flocks = 233
Figure (1)

USDA







Scrapie – Impact on Industry

- The American Sheep Industry Association has estimated that Scrapie is costing U.S. producers $20-25 million annually

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Scrapie - Impact on Industry

- Infected flocks are less productive.
  - Affected animals typically die during their peak productive years
  - Infected flocks loose markets for breeding animals and often spread the disease before it is known that the flock is infected

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Scrapie - Impact on Industry

- The presence of scrapie in the U.S. limits the export of breeding stock, semen, and embryos to many other countries.

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Scrapie - Impact on Industry

- Recent attention to TSEs due to BSE and nvCJD and the possible link between BSE and the feeding of scrapie infected sheep derived meat and bone meal to cattle in England has resulted in:

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Scrapie - Impact on Industry

- Many renderers have declined to render sheep offal and to pick up dead sheep causing packers and producers to incur significant disposal costs,

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Scrapie - Impact on Industry

- Other countries have expressed concerns and have indicated that they may prohibit or restrict certain ruminant products because the U.S. has scrapie, and

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Scrapie - Impact on Industry

- Our domestic and international markets for sheep derived meat and bone meal, have been adversely affected.

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Important Considerations:

- **Scrapie is an infectious and contagious disease**
- **Animals do not recover from scrapie.**
- **The incubation period is typically between 2 and 5 years for animals exposed at or near birth**

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Important Considerations:

- **The incubation period is typically between 6 and 9 years for animals exposed post weaning**
- **Animals exposed at or near birth are most susceptible to scrapie.**
- **The only tissue/fluid that leaves the body of a live animal that has been shown to contain infectious material is the placenta and birth fluids.**

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Important Considerations:

- The infectious agent is hard to destroy.
- Rams are believed to be of low risk for spreading scrapie
- Artificial insemination does not transmit scrapie.

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Important Considerations:

- Embryo transplant appears to reduce the risk of scrapie transmission from donor to offspring; however, there are conflicting reports in the literature.

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Important Considerations:

- Genotype plays a strong role in the development of clinical disease but its effect on infection and transmission has not been fully determined.

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Genetics

- most susceptible to less susceptible
- Codon 171
  - QQ, HH, QR, HR, RR
- Codon 136
  - VV, AV, AA

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Eradication Program Goals

- Eliminate outbreaks of scrapie by 2010.
- Have the U.S. officially free by international standards by 2017.

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Eradication Program Goals

- Minimize ongoing losses to the sheep industry and make it more competitive in the global market.
- Mitigate impacts on international trade of all ruminant products.

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Intermediate Goals

- Develop effective scrapie control and surveillance programs in all States
- Educate the industry about scrapie, its control, and the eradication effort

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Intermediate Goals

- Determine the national & regional prevalence of scrapie
- Develop effective slaughter surveillance with trace back to flock of origin

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Interstate Movement Final Rule

- Published August 21, 2001
- Effective:
  - 9/20/01 – movement for exposed animals and most consistent State requirements
  - 11/19/01 - for most ID requirements
  - 2/19/02 – for breeding whiteface sheep under 18 months of age

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Identification Requirements for Interstate Movement

Official Identification System

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What is the Purpose of Identifying Sheep and Goats?

- Purpose
  - To enable traceback of scrapie-positive animals to their flock or herd of origin, and
  - Trace out of animals from infected and source flocks

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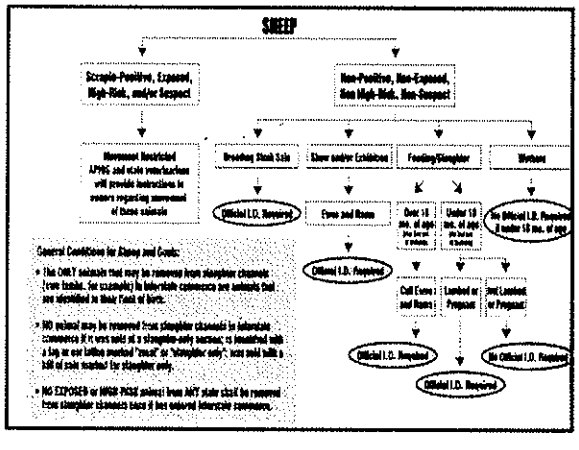

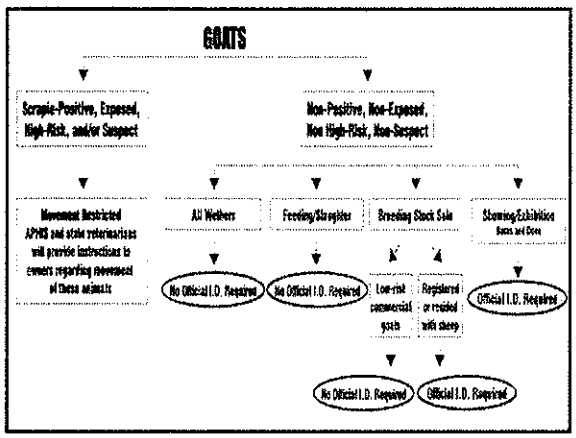

What types of identification may be used?

- Types of Official Identification
  - Official Eartags
    - USDA provided eartags
    - USDA approved eartags
  - Electronic implants (SFCP)
  - Registry Tattoos (certificate needed)
  - Premises ID number tattoos
  - Official backtags for animals moving directly to slaughter

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What types of tags can be used?

- Premises Based Individual Identification Tags
  - APHIS provided or owner purchased tags produced by APHIS approved tag companies
- Individual Identification Tags
  - USDA provided serial number tags

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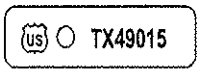
What do Premises Based Tags Look Like?

Premises Tags with Individual Animal Identification Numbers

Plastic Rotary Tags

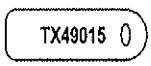
Female Part



Male Part

Metal Tags

Back



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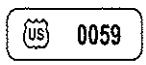

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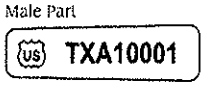
What do Serial Number Tags Look Like?

Tags with Unique Serial Numbers

Plastic Rotary Tags

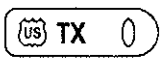
Female Part

Male Part



Metal Tags

Back



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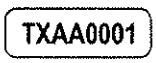

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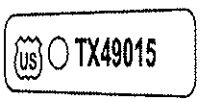
What do Premises/Serial Number Tags Look Like?

Premises Tags with Unique Serial Numbers

Plastic Rotary Tags

Female Part



Male Part

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When can premises only ID be used?

- premises only identification such as registered brands and ear notches are limited to uses such as grazing and low-risk commercial flocks

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Who Must Apply Identification and When?

- The owner of a flock is responsible for the identification of the animal prior to commingling it in interstate commerce.

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Can I move an animal intrastate without ID?

- States determine intrastate requirements;however, all States have agreed to implement intrastate ID within 2 years.
- Also if an animal has changed hands in intrastate commerce such that it can not be identified to its flock of origin and in some cases birth, it can not be moved interstate with some exceptions.

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Who will be held responsible for unidentified animals under the federal regulations?

- Any person who delivers or receives an unidentified animal that is required to be identified at a place where it will be commingled with animals in interstate commerce.

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Who will be held responsible for unidentified animals under the federal regulations?

- Any person who removes an unidentified animal that is required to be identified from a site were commingling has occurred with animals in interstate commerce.

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Sheep and Goat Identification Tutorial

How to Comply with the Identification Requirements

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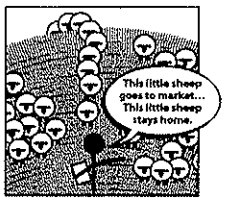
How to Comply with the Identification Requirements

- Do your animals need ID to move interstate?



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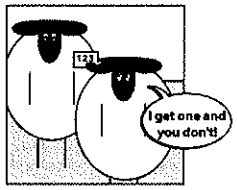
Step 1 - Determine which animals need ID

- Determine which animals need official eartags or other official ID to move interstate



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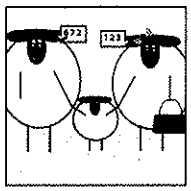
Determine which animals need ID (Cont.)



- Breeding Ewes or Rams
  - If going to show: Official ID required
  - If going to sale: Official ID required
  - If staying at home: No official ID required
- A show/exhibition is considered interstate commerce if out of State animals are allowed at the show

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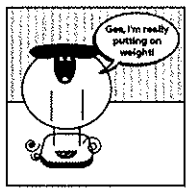
Determine which animals need ID (Cont.)



- Fat lambs
  - Lambs under 18 months of age going to slaughter: No Official ID required
  - Ewe lambs under 18 months of age leaving slaughter channels: Official ID required

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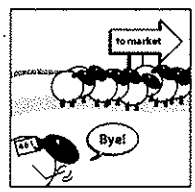
Determine which animals need ID (Cont.)



- Culled sheep
  - Culled Ewes or Rams: Official ID required
  - Cull sheep defined as greater than 18 months of age

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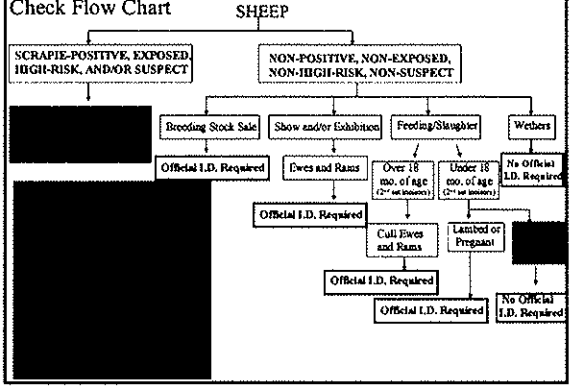
Different class of sheep? Check Flow Chart

SCRAPIE ERADICATION PROGRAM
SHEEP IDENTIFICATION REQUIREMENTS



Step 2 – Request Premises Identification Number

- If identification is needed-Call local area office or 1-866-USDA-TAG (866-873-2824) and request a flock or herd ID number
  - Premises numbers will be assigned by the Area VS office and/or the State Veterinarian's office in each State.

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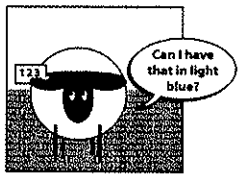
Step 3- Obtaining Ear Tags



- You can request official USDA tags free from your local APHIS office 1-866-USDA-TAG
- APHIS provided tags will shipped direct to the producer by the tag company or distributed by the office.
- Sorry free tags come only in white ⊗

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Obtaining Ear Tags (Cont.)

- You can purchase a variety of ear tag styles and colors from an approved tag company
  - If you are interested in these tags please request the list of approved tag companies when requesting your premises ID number.

*Note: Yellow and red Metal tags are reserved for scrapie-exposed and scrapie-positive animals respectively.

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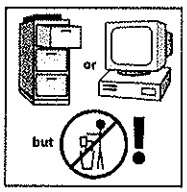
Step 4- Record Keeping



- Set up a method to record the eartags or other official ID you applied
- When an animal is sold you must provide the buyer with flock of origin information

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Record Keeping Sellers (Cont.)

- Record number of animals sold and their premises identification number or their individual numbers
- Record the date of sale
- Record name, address and phone number of buyer

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Record Keeping Buyer (Cont.)

- Record number of animals purchased and their premises of origin identification number or their individual numbers
- Record the date of purchase
- Record name, address and phone number of the seller
- for breeding or exhibition animals record the flock of birth ID number if different from the flock of origin ID number

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Record Keeping (Cont.)

- A record form is available to you that includes the minimum requirements

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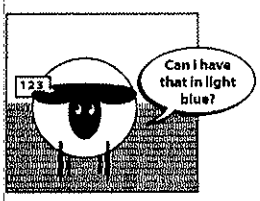
Step 5- Apply ID tags

- Apply tags to the animals before they leave your farm



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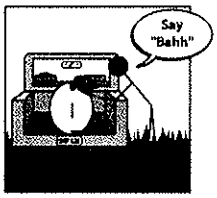
Step 6 – Certificate of Veterinary Inspection (Health Certificate)



- Prior to interstate shipment or sale in interstate commerce of breeding or exhibition animals you must obtain a certificate of veterinary inspection (health certificate) from an accredited veterinarian
- Certificate must be dated no more than 30 days prior to the movement or sale

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Step 7- Retain Records

- You must keep records for at least 5 years from the time the animals are transported or sold

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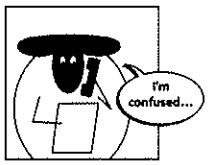
Step 8 – Questions?

- Call 1-866-USDA-TAG (866-873-2824) for any questions



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Interstate Movement Restrictions

Prohibited Movement

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Prohibited Movement

- Note:
  - Most animals that are prohibited movement are eligible for indemnity

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Prohibited Movement

- Scrapie-positive and suspect animals
  - Prohibited in every case

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Prohibited Movement

- Sexually Intact high-risk animals for breeding or exhibition

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Prohibited Movement

- Non-high-risk animals from infected or source flocks/herds
  - Movement for breeding purposes prohibited except certain animals in flocks enrolled in a pilot project which must meet the requirements for exposed animals.

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Prohibited Movement

- Breeding animals from noncompliant flocks or herds
  - Movement for breeding purposes prohibited in every case
  - Note: animals from noncompliant flocks are considered "exposed"

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Interstate Movement Restrictions

Restricted Movement

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Restricted Movement *High Risk Animals

- Sexually Intact high-risk animals to slaughter
  - Official individual ID and a permit or
  - A permit and an indelible "S" on left jaw or
  - A sealed conveyance and a permit

*Note: This includes sexually intact animals from infected or source flocks that are not scrapie-positive or suspect

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Restricted Movement *Exposed animals

- For Breeding
  - Official individual ID and a permit and
  - For any female, the result of an official genotype test must be included or attached to the permit
  - Must be QR or RR at codon 171
  - If born after 1/1/2001, permit must include flock of birth or origin

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Restricted Movement *Exposed animals

- For Exhibition
  - Same as for breeding
  - An owner and veterinarian statement that animal not lambed or aborted within 30 days of exhibition and animal is not due to lamb within 30 days
  - No visible vaginal discharge

1/23/02 69

Restricted Movement *Exposed animals

- For Slaughter (under 18 months)
  - Official individual ID for any animal not moving direct to slaughter or terminal feedlot.
- For slaughter (over 18 months of age)
  - Official individual ID

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Restricted Movement *Exposed animals

- For Grazing
  - Official individual ID and a permit
  - For any female sheep the result of an official genotype test must be attached
  - Must be QR or RR at codon 171

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Consistent State Requirements
(That may Directly Impact Producers)

- To be a Consistent State, the State must require reporting of scrapie suspects and quarantine infected and source flocks

1/23/02 72

Consistent State Requirements to be met within 2 years

- Official identification, upon change of ownership, of
  - all breeding animals of any age and
  - any sheep over 18 months of age (as evidenced by eruption of the second incisor such that the animal may be traced to its flock of birth.
- States may give exemptions to certain low ris populations such as commercial goats when n evidence of scrapie has been found in these populations in the State.

1/23/02 73

Control of Scrapie-Flock Clean-up

- **Indemnity**
  - **APHIS will provide:**
    - **Indemnity for high-risk, suspect, and scrapie-positive animals when owners agree to participate in flock or herd clean-up**
    - **Value is determined based on AMS posted prices using the lotus 1-2-3 calculator.**

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Control of Scrapie-Flock Clean-up

- **APHIS will also provide:**
  - **Live-animal testing**
  - **Official genotyping**
  - **Monitoring of the flock/herd**
  - **Necropsy of mature animals that die**

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Scrapie – Surveillance

- **A Scrapie Ovine Slaughter Surveillance Study is currently being conducted to estimate national and regional prevalence of scrapie in mature cull sheep population**
- **A live animal test has been developed and will be used by APHIS to test exposed animals and to assist infected flocks with flock cleanup plans. Wyoming may pilot the test for surveillance.**

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SUCCESS DEPENDS on All of US!

- Active participation by Producers, Markets and Dealers as well as State an Federal government commitment and cooperation are critical for success.
- Scrapie Eradication will not be easy and it will not be immediate. We must b prepared for a large and sustained effort.

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SECTION III
MANAGEMENT SECTION

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43rd ANNUAL SHEEP DAY

HETTINGER RESEARCH EXTENSION CENTER
HETTINGER, NORTH DAKOTA

FEBRUARY 13, 2002

FLOCK CALENDAR OUTLINE

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

PRIOR TO BREEDING

1. Bag and mouth ewes and cull those that are not sound.
2. Replace culled ewes with top-end yearlings or ewe lambs.
3. Keep replacement ewe lambs on growing ration.
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 the breeding season. Flush rams in poor condition.
   c. Allow at least two mature rams (preferably three) or four buck lambs per 100 ewes.
   d. Utilize production records to evaluate anticipated breeding ability.
5. Flush ewes:
   a. One pound grain/day two to five weeks before breeding (usually 17 days).
   b. If ewes are over-conditioned, the effect of flushing will be lessened.
6. Vaccinate ewes for vibriosis and enzootic abortion (EAE).
7. Identify all ewes and rams with ear tags, paint brands or tattoos.

BREEDING

1. The ovulation rate of a ewe tends to be lowered at the first part of the breeding season. Vasectomized or teaser rams run with the ewes through the first heat period tend to stimulate them and increase the ovulation rate at the second heat period.
2. Use a ram marking harness or painted brisket to monitor breeding. Soft gun grease with 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 heat cycles or 68 days may be beneficial.
4. Remove rams from ewes after the season (don't winter rams with ewes).

PRIOR TO LAMBING — EARLY PREGNANCY (First 15 Weeks)

1. Watch general health of ewes. If possible sort off thin ewes and give them extra feed so they can catch up.

2. Feed the poor quality roughage you have on hand during this period, saving the better for lambing.

3. An exception to the above is feeding pregnant ewe lambs. They should receive good quality roughage and grain (about 20 percent of the ration) during this period.

LAST SIX WEEKS BEFORE LAMBING

1. Trim hooves and treat for internal parasites.

2. Six to four weeks before lambing feed 1/4 to 1/3 pound grain/ewe/day.

3. Shear ewes before lambing (with highly prolific ewes at least a month before is preferred). Keep feeding schedule regular and watch weather conditions immediately after shearing (cold).

4. Vaccinate ewes for enterotoxemia.

5. Control ticks and lice immediately after shearing.

6. Four weeks before lambing increase grain to ½ 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** — Don't do both.

9. Check facilities and equipment to be sure everything is ready for lambing.

10. Two weeks before lambing increase grain to 1 pound per ewe per day.

LAMBING

1. Be prepared for the first lambs 142 days after turning the rams in with the ewes, 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. Put ewe and lambs in lambing pen (jug) after lambing (not before).

4. Grain feeding the ewes during the first three days after lambing is **not** necessary.

5. Be available to provide assistance if ewe has troubles.

6. Disinfect lamb's navel with iodine as soon after birth as possible.

7. Be sure both teats are functioning and lambs nurse as soon as possible.

8. Use additional heat sources (heat lamps, etc.) in cold weather.

9. Brand ewe and lambs with identical number on same sides. Identify lambs with ear tags, tattoos or both.

10. Turn ewe 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 the best success. Few ewes can successfully nurse more than two lambs.

END OF LAMBING TO WEANING

1. Feed ewes according to number of lambs suckling. 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 the 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 or when they weigh at least 40 pounds and are eating creep and drinking water. The advantage of early weaning is that the ewe's milk production drops off to almost nothing after eight weeks of lactation.

3. Grain 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 the ewe following weaning lessens the chance of mastitis to occur. Poorer quality roughage should be fed to the ewes for at least 10 to 14 days following weaning.

4. Handle the ewes as little as possible for about 10 days following weaning. Tight udders bruise easily. If possible, bed the area where the ewes will rest heavily with straw to form a soft bed for the ewes to lay on.

WEANING TO PRE-BREEDING

1. If ewes go to pasture, treat for internal parasites.

2. Feed a maintenance ration to the ewes. Put ewe lambs that lambed back on a growing ration once they have quit milking.

3. Adjust ewe's conditions 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 this decision early. Relatively weak lambs remaining with the ewes can experience more stress than those reared artificially. Consider the following tips:

- It is essential that newborn lambs receive colostrum milk. Cow's colostrum will work if ewe's milk is not available. Do not dilute with water or warm too quickly if colostrum is frozen.
- Lambs should be removed from sight and hearing distance of ewe.
- 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.
- Use good equipment. Self priming nipple and tube assemblies have been found to be excellent for starting lambs.
- 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 fed cold milk grow well with less problems from scours and other digestive disturbance. Cold milk keeps better too.
- There is a Formaldehyde solution commercially available that retards bacterial growth in milk (1 cc/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 not be able to obtain 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 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 weeks of age as it encourages bloat. Caution! Do not feed leafy alfalfa until two weeks after weaning, as it may encourage bloat.
- Wean lambs abruptly at 21-30 days of age. When to wean depends upon whether lambs are eating creep feed and drinking water. Newly weaned lambs will go backwards for several days. Don't be alarmed, they will make compensating gains later on.

SHEEPBARNS AND EQUIPMENT PLANS

Timothy C. Faller
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North Dakota State University

NOTE: These and other plans are available through county agents or from Extension Agricultural Engineering, NDSU, Fargo, ND. The drawings show construction details and include a materials list for estimating. Due to changes in lumber sizes, lumber grades, plywood quality, and other developments in building materials, some adjustments are required for older plans. (Present charge is shown or $1.00 per sheet.)

CORRALS AND BARNS

Plan No.	Plan Title	Sheets
MW 72050	Pole Utility Buildings	$2.00
MW 72505	Slatted Floor, 40'x72', Feeder Lamb Barn	3.00
MW 72506	240 Ewe and Lambing Barn, 40'x104'	3.00
MW 72507	500 Ewe and Lamb Feeding Barn, 74'x256'	3.00
MW 72508	12' x 16' Portable Lamb Feeding Shed	2.00
MW 72509	40 Ewe and Lambing Barn, 24x32'	2.00
ND Plan	Confinement Sheep Barn & Hay Storage (at Hettinger)	1.00
Reprint #759	Practical Sheep Housing for North Dakota	No Charge
USDA 6096	Shearing Shed & Corral Arrangement	1
USDA 6236	Portable Handling Corral for Sheep (Metal Wood)	1
AE-683	Sheep Barn Layout	No Charge
AED-13	Insulation and Heat Loss	No Charge
AED-19	Slip Resistant Concrete Floors	No Charge
AED-25	Earth Tube Heat Exchange System Planning	No Charge
MWPS-3	Sheep Housing and Equipment Handbook (This 90 page booklet was revised in 1994. It includes barn and layout planning plus plans for fences and sheep equipment.)	10.00
MWPS-9	Designs for Glued Trusses	5.00

FEED HANDLING & FEEDERS

USDA 5917	Fencing, Feeding, and Creep Panels	1
Reprint #409	Chopped Hay Feeder for Sheep	No Charge
Reprint	16 ft. Collapsible Fenceline Feedbunk for Sheep	No Charge
ND 872-1-1	Stationary Roughage Self Feeder for 70 Ewes or 160 Lambs	No Charge
ND 872-1-2	Portable Roughage Self Feeder for 40 Ewes or 80 Lambs	No Charge

Plan No.	Plan Title	Sheets
MW 73110	24 ft. wide Clearspan Pole Frame Hay Shed	$ 3.00
MW 73111	36 ft. wide Clearspan Pole Frame Hay Shed	3.00
MW 73112	48 ft. wide Clearspan Pole Frame Hay Shed	3.00
MW 73113	32 ft. & 48 ft. Wide Pole Frame Hay Shed (Interior Poles)	3.00
MW 73210	Moveable Grain Storage Walls, 6' to 12' High	2.00
MW 73217	20, 45, 170, and 340 Bu. Hoppered Grain Bins	3.00
MW 73220	48 ft. Wide Pole Frame Grain Storage	2.00
MW 73250	Grain Storage Buildings, 600, 1000, 1200, 1500 or 2000 Bu.	3.00
MW 73293	Grain-Feed Handling Center, Work Tower Across Drive	4.00
MW 73294	Grain-Feed Handling Center, Work Tower Beside Drive	4.00
APA	10 Ton Hoppered Feed Bin	No Charge
APA	4 Compartment Bin for Feed Mill	No Charge
AED-15	Horizontal Bunker Silos, Concrete Tilt-up	No Charge
USDA 6090	5500 Bushel Wooden Grain Bin	2
MWPS-13	Planning Grain-Feed Handling Handbook	5.00