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Technical Note: Effects of Supplementation of Expired Human Foodstuffs on Intake and Digestion by Wethers Fed a Base Diet of Grass Hay and Alfalfa/Barley Pellets

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Summary

There is potential for expired human foodstuffs to be used as an energy supplement for livestock. Sixteen crossbred wether lambs were used in a completely randomized design to investigate the effects of feeding supplemental expired human foodstuffs on DM, OM, ADF and NDF digestibility, and intake. Wethers were fed (DM basis) isocaloric amounts of the following treatments: whole barley served as the control (**BAR**: 0.20 kg·wether^{-1}·d^{-1}), potato chips (**PC**: 0.15 kg·wether^{-1}·d^{-1}), macaroni (**MAC**: 0.21 kg·wether^{-1}·d^{-1}), and donuts (**DON:** 0.15 kg·wether^{-1}·d^{-1}). Wethers were fed 0.60 kg·wether^{-1}·d^{-1} alfalfa/barley pellets and allowed ad libitum access to chopped hay. Wethers were placed in confinement crates for a 7-d acclimation period, fitted with fecal bags on d 0 and fed twice daily. Following acclimation, daily intakes, refusals, and fecal outputs were used to determine DM, OM, fiber digestibility and intake. Measures of intake and digestibility did not differ ($P > 0.23$) among treatments. It is concluded that these expired human foodstuffs have the potential to be used in ruminant diets as an alternative to traditional feedstuffs.

Key Words: Digestibility, Expired Foods, Intake.

Introduction

Every year, large quantities of retail food products are removed from the supply chain because they have expired. In 2001, more than $900 million of expired food was wasted (GMA, 2002). Forty percent of food in the United States goes uneaten, which is the equivalent of throwing $165 billion dollars into landfills (NRDC, 2012). Wasted and unused foods are a major substrate source for methane production from landfills. In the United States, methane emissions from landfills are equal to approximately 125 Tg CO_2 Eq., ranking third behind natural gas systems and enteric fermentation (EPA, 2013). In the last 10 years, barley, a common traditional energy supplement for livestock, has increased in price by 124 percent (NASS, 2012). As an alternative to expensive energy supplements, expired human foodstuffs may have the potential to be part of a ration for livestock, as well as provide an environmentally friendly method of disposal. Previous research has found negative effects of starch supplementation on fiber digestion and DMI of low-quality forages (Hoover, 1986; Chase and Hibberd, 1987). However, low proportions of starch supplementation can potentially increase dry-matter intake and digestion (Pordomingo, et al., 1991). Supplementing ruminants with fat has been reported to depress digestibility but not impact forage OM intake (Brokaw et al., 2001). Sources of supplemental energy traditionally have included grains, readily digestible-fiber sources, and high-quality forages. The effects of energy supplementation on intake and digestibility have been variable (Caton and Dhuyvetter, 1997), and little information is available on the impacts of feeding expired human foodstuff on intake and digestibility by small ruminants. The objective of this study was to compare daily intakes and digestibility of DM, OM, ADF, and NDF of sheep fed chopped hay and alfalfa/barley pellets and supplemented with expired human foodstuffs (macaroni, potato chips, and donuts).

Materials and Methods

All animal use procedures were approved by the Montana State University Animal Care and Use Committee (Protocol #1144). Sixteen crossbred

Table 1. Analysis of dietary components[1]

Item	Chopped hay	Alfalfa/ barley pellet	Barley	Donuts	Macaroni	Potato Chips
DM, %	92.1	96.2	91.7	83.8	92.7	97.9
OM, %	91.2	89.5	98.6	98.1	99.1	94.9
NDF, %	58.8	48.2	20.3	5.4	8.4	3.8
ADF, %	40.5	37.6	16.9	4.7	7.6	3.0
CP, %	9.0	16.8	10.6	5.2	13.0	7.9
EE[2, 3], %	1.0	1.3	1.8	28.5	1.7	33.0
TDN[2, 4], %	58.1	68.3	88.0	118.7	85.4	119.4

[1] All values presented on DM basis. Dietary composition was determined by analyzing subsamples collected and composited throughout the trial. Accuracy was ensured by adequate replication with acceptance of mean values that were within 5% of each other.
[2] Values for Donut, Macaroni, and Potato Chips calculated based on energy values from National Nutrient Database for Standard Reference (USDA, 1999). Values for Chopped hay and Alfalfa/barley pellets taken from NRC (1985).
[3] EE= ether extract.
[4] TDN, %= (0.5 x %Crude Fiber) + (0.90 x %Nitrogen-Free Extract) + (0.75 x %Crude Protein) + (2.25 x 0.90 x %Ether Extract)

Table 2. Values used to develop isocaloric diets that approximate energy needs and projected DMI for a 40 kg wether

Ingredient	Treatment diets[1]			
	BAR[2]	DON[3]	MAC[4]	PC[5]
	amount fed, kg; (amount TDN in amount fed, kg)			
Chopped hay	0.70; (0.39)	0.70; (0.39)	0.70; (0.39)	0.70; (0.39)
Alfalfa/barley pellet	0.60; (0.41)	0.60; (0.41)	0.60; (0.41)	0.60; (0.41)
Barley	0.20; (0.18)			
Donut		0.15; (0.18)		
Macaroni			0.21; (0.18)	
Potato chip				0.15; (0.18)
Projected DMI	1.50[6]	1.45	1.51	1.45
Projected TDN intake[7]	0.98	0.98	0.98	0.98

[1] Calculated (Alfalfa/barley pellet, Barley, Donut, Macaroni, Potato chip) and projected (chopped hay) intake to equal treatment isocaloric diets
[2] Fed barley at 0.20 kg·wether^{-1}·d^{-1}, alfalfa/barley pellets at 0.60 kg·wether^{-1}·d^{-1}, and ad libitum access to chopped hay.
[3] Fed donuts at 0.15 kg·wether^{-1}·d^{-1}, alfalfa/barley pellets at 0.60 kg·wether^{-1}·d^{-1}, and ad libitum access to chopped hay.
[4] Fed macaroni at 0.21 kg·wether^{-1}·d^{-1}, alfalfa/barley pellets at 0.60 kg·wether^{-1}·d^{-1}, and ad libitum access to chopped hay.
[5] Fed potato chips at 0.15 kg·wether^{-1}·d^{-1}, alfalfa/barley pellets at 0.60 kg·wether^{-1}·d^{-1}, and ad libitum access to chopped hay.
[6] Based on NRC (1985) estimated DMI for a 40 kg wether.
[7] Based on NRC (1985) estimate of 1.16 kg of TDN to support moderate growth of a 40 kg wether x 0.85 to prevent any potential of digestive upset associated with high energy, highly soluble carbohydrate feeds.

wether lambs (Suffolk/Hampshire x Western white face; 6-mo-old; BW = 38±2 kg) were used in a completely randomized design to investigate the effects of feeding supplemental expired human food on the intake and digestibility of treatment diets.

Treatments were: barley fed at 0.20 kg·wether^{-1}·d^{-1} (**BAR**), potato chips fed at 0.15 kg·wether^{-1}·d^{-1} (**PC**), macaroni fed at 0.21 kg·wether^{-1}·d^{-1} (**MAC**), and donuts fed at 0.15 kg·wether^{-1}·d^{-1} (**DON**), all on a DM basis. Treatments were formulated to be isocaloric based on NRC (1985) estimated TDN values for barley, brome grass hay, and alfalfa, and calculated TDN values of macaroni, donuts, and potato chips based on estimated nutrient content (USDA, 1999) (Tables 1 and 2). The BAR diet was formulated to contain 20 percent barley and provide 85 percent of the TDN requirement for a 40 kg wether consuming 1.5 kg of DM (NRC 1985). All other treatment diets were then formulated to be equal in energy to the BAR diet. Diets were formulated to provide 85 percent of NRC (1985) TDN requirements to insure no digestive problems associated with highly soluble, carbohydrate feeds. Wethers were allowed ad libitum access to chopped-grass hay and fed 0.60 kg·wether^{-1}·d^{-1} of an 80- percent alfalfa, 20-percent barley pellet (DM basis) to insure adequate CP intake.

Wethers were housed in metabolism crates (75 cm x 125 cm), fitted with fecal bags at the beginning of the acclimation period, and allowed a 7-d period to acclimate to diets and environment. The study took place under 24 h light. Wethers were offered total respective treatments and a half ration of chopped hay (60 percent of previous day's intake) and 0.30 kg·wether^{-1}·d^{-1} of alfalfa/barley pellets at 0600 h. The remaining chopped hay (60 percent of previous day's intake) and 0.30 kg·wether^{-1}·d^{-1} of alfalfa/barley pellets were fed at 1600 h. Feed samples were taken daily, and each feedstuff was compiled over the 7-d period for later analysis. Feed offered and feed refused for each wether were weighed and subsampled every 24-h and used to calculate DMI. Feces were collected from fecal bags twice daily, composited by animal, and stored for later weighing and analysis. At the end of the 7-d trial, total fecal weights for each lamb were recorded, and a subsample of feces was gathered and composited by animal for determination of nutrient component analyses, DMI, OM intake, DM digestibility, NDF digestibility, and OM digestibility *in vivo*.

Treatment samples, chopped hay, and alfalfa/barley pellets were dried in a 60° C forced-air oven and ground to pass through a 1-mm screen in a Wiley mill. Samples were analyzed for DM, OM, Kjeldahl N (AOAC, 1984), NDF, and ADF (Van Soest et al., 1991; Table 1). Values for EE (ether extract) and TDN were obtained from the National Nutrient Database for Standard Reference (USDA, 1999). Fecal samples were analyzed for DM, OM, Kjeldahl N (AOAC, 1984), NDF and ADF (Van Soest et al., 1991).

Daily intakes and digestibility of DM, OM, ADF, and NDF were calculated. Data were analyzed using the GLM procedure of SAS (SAS Inst. Inc., Cary, N.C.), with expired feedstuff as the fixed effect. Animal was considered the experimental unit. Differences among treatments were considered significant at $P < 0.10$.

Results and Discussion

Three wethers were removed from the trial during the adaptation period, two from the MAC treatment and one from the PC treatment, due to inability to adjust to the research environment. Daily intakes of each treatment ingredient, alfalfa/barley pellets, and chopped hay are presented in Table 3. Wethers consumed all of the alfalfa/barley pellets and treatment ingredients provided. Daily intake of each feedstuff was compiled by wether and multiplied by the feedstuff nutrient profile and presented in Table 4. Total diet digestibility of DM, OM, NDF, and ADF is presented in Table 5.

Measures of intake and digestibility did not differ ($P > 0.23$) among treatments. Chase and Hibberd (1987) reported that providing 1, 2, or 3 kg/d ground corn to mature beef cows consuming low-quality, native-grass hay resulted in a linear decrease in cellulose digestibility and hay intake. Pordomingo et al. (1991) found that supplementing steers grazing low-quality native range with 0.20 percent of BW of corn increased OM intake and *in situ* OM digestion. These authors also reported that steers supplemented with 0.40 percent and 0.60 percent of BW of corn experienced a decrease in OM intake and *in situ* OM digestibility (Pordomingo et al., 1991). Furthermore, Bodine et al. (2000) suggested that barley contains less starch and more degradable-intake protein (DIP) than corn, which may

Table 3. Actual amounts of feed ingredients consumed by wethers with ad libitum access to chopped hay and supplemented with isocaloric treatments and alfalfa/barley pellets (0.60 kg · wether^{-1} · d^{-1})[1]

Item	BAR[2]	DON[3]	MAC[4]	PC[5]	SE
No. of wethers	4	4	2	3	-
Treatment DMI, kg[6]	0.20	0.15	0.21	0.14	-
Alfalfa/barley pellet DMI, kg[6]	0.60	0.60	0.60	0.60	0.000
Chopped hay DMI, kg[6]	0.34	0.43	0.50	0.41	0.056

[1] All values presented on DM basis. Dietary composition was determined by analyzing subsamples collected and composited throughout the trial. Accuracy was ensured by adequate replication with acceptance of mean values that were within 5% of each other.
[2] Fed barley at 0.20 kg·wether^{-1}·d^{-1}, alfalfa/barley pellets at 0.60 kg·wether^{-1}·d^{-1}, and ad libitum access to chopped hay.
[3] Fed donuts at 0.15 kg·wether^{-1}·d^{-1}, alfalfa/barley pellets at 0.60 kg·wether^{-1}·d^{-1}, and ad libitum access to chopped hay.
[4] Fed macaroni at 0.21 kg·wether^{-1}·d^{-1}, alfalfa/barley pellets at 0.60 kg·wether^{-1}·d^{-1}, and ad libitum access to chopped hay.
[5] Fed potato chips at 0.15 kg·wether^{-1}·d^{-1}, alfalfa/barley pellets at 0.60 kg·wether^{-1}·d^{-1}, and ad libitum access to chopped hay.
[6] Mean daily DMI over 1 wk trial.

Table 4. Feed components[1] consumed by wethers with ad libitum access to chopped hay and supplemented with isocaloric treatments and alfalfa/barley pellets (0.66 kg · wether^{-1} · d^{-1})

Item	BAR[2]	DON[3]	MAC[4]	PC[5]	SE
No. of wethers	4	4	2	3	-
DM, kg[6]	1.150	1.190	1.320	1.150	0.068
OM, kg[6]	1.052	1.087	1.210	1.041	0.062
NDF, kg[6]	0.533	0.551	0.604	0.536	0.041
ADF, kg[6]	0.399	0.408	0.446	0.397	0.023

[1] All values presented on DM basis. Dietary composition was determined by analyzing subsamples collected and composited throughout the trial. Accuracy was ensured by adequate replication with acceptance of mean values that were within 5% of each other.
[2] Fed barley at 0.20 kg·wether^{-1}·d^{-1}, alfalfa/barley pellets at 0.60 kg·wether^{-1}·d^{-1}, and ad libitum access to chopped hay.
[3] Fed donuts at 0.15 kg·wether^{-1}·d^{-1}, alfalfa/barley pellets at 0.60 kg·wether^{-1}·d^{-1}, and ad libitum access to chopped hay.
[4] Fed macaroni at 0.21 kg·wether^{-1}·d^{-1}, alfalfa/barley pellets at 0.60 kg·wether^{-1}·d^{-1}, and ad libitum access to chopped hay.
[5] Fed potato chips at 0.15 kg·wether^{-1}·d^{-1}, alfalfa/barley pellets at 0.60 kg·wether^{-1}·d^{-1}, and ad libitum access to chopped hay.
[6] Mean daily DMI over 1 wk trial.

Table 5. Diet digestibility of wethers fed alfalfa/barley pellets (0.66 kg · wether^{-1} · d^{-1}), expired human foodstuffs, and chopped hay (ad libitum access)

Item	BAR1	DON2	MAC3	PC4	SE
No. of wethers	4	4	2	3	-
Digestibility (%)					
DM	74.7	69.8	67.3	71.4	2.40
OM	76.1	71.1	69.0	72.6	2.32
NDF	62.9	58.1	59.3	63.8	4.02
ADF	62.4	55.0	52.7	59.1	4.24

[1] Fed barley at 0.20 kg·wether^{-1}·d^{-1}, alfalfa/barley pellets at 0.60 kg·wether^{-1}·d^{-1}, and ad libitum access to chopped hay.
[2] Fed donuts at 0.15 kg·wether^{-1}·d^{-1}, alfalfa/barley pellets at 0.60 kg·wether^{-1}·d^{-1}, and ad libitum access to chopped hay.
[3] Fed macaroni at 0.21 kg·wether^{-1}·d^{-1}, alfalfa/barley pellets at 0.60 kg·wether^{-1}·d^{-1}, and ad libitum access to chopped hay.
[4] Fed potato chips at 0.15 kg·wether^{-1}·d^{-1}, alfalfa/barley pellets at 0.60 kg·wether^{-1}·d^{-1}, and ad libitum access to chopped hay.

lessen the negative associative effects observed with cereal supplementation of low-quality forage diets. Compared to the BAR control treatment in our study, none of the expired foods impacted either measures of intake or digestibility when fed at an equivalent of 20 percent barley in the diet or the barley equivalent of 0.50 percent of BW.

Our results are in contrast to those presented by Champe and Church (1980), who found increasing digestibility of all dietary components by lambs fed increasing supplementation (0 percent, 20 percent and 40 percent of diet) of a commercially available dried bakery product. Champe and Church (1980) found no impact on daily DMI by lambs with increasing proportions of supplemental dried bakery product. It is important to note the difference in nutritional composition of the dried bakery product used by Champe and Church (1980) and the DON and PC used in this study, specifically fat content (8.5 percent and 28.5 percent, respectively). Previous research has demonstrated deleterious effects of fat on nutrient digestibility by cattle consuming forage diets. Brokaw et al. (2001) reported that heifers on high-forage diets that were supplemented daily (0.3 percent of BW) with a mixture containing cracked corn, corn gluten meal, and soybean oil (12.5 percent of supplemental DM; Oil) experienced depressed OM and NDF digestibility compared to heifers supplemented with conventional corn. The authors went on to report that forage OM intake was not affected by feeding supplemental fat at 1.5 percent to 1.74 percent of diet DM (Brokaw et al., 2001). Rahnema and Borton (2000) reported that substituting 15 percent to 20 percent of corn with potato chip scraps in nursery pig diets decreased DMI but had no effect on ADG and improved the gain:feed ratio. The PC supplement contained the most fat (Table 1) of all the treatments, but did not negatively impact digestibility.

Nutrient variation for by-product feeds, such as those examined in this study, can be considerable depending on factors, such as source, basal ingredients, and manufacturing processes. Arosemena et al. (1995) reported that the average nutrient composition of nine commonly used by-product feeds differed by more than 20 percent from tabular NRC values. Furthermore, the authors stressed that accurate nutrient analyses of these ingredients became more critical as their concentration in the diet was increased. Additionally, the potential associative effects of the by-product feeds examined in this study with the forage provided are unknown.

Conclusions

In our study, no particular treatment stood out as superior or inferior in digestibility or DMI compared to BAR. Expired human food products (macaroni, potato chips, and donuts) did not impact intake of low-quality forage or measures of digestibility. Using expired human foodstuffs as an energy supplement for livestock may be an expense-saving opportunity for producers, as well

as a favorable disposal option for otherwise wasted products. Increasing production costs challenge livestock producers to investigate novel sources of feed. Expired human foodstuffs could provide an economical alternative to traditional energy supplements, without substantial negative impacts on intake or digestibility. Further studies in larger numbers of animals in different stages of production are warranted.

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Tail Length at Docking and Weaning of Lambs

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Summary

This study was conducted with crossbred lambs (n = 109 female and 120 male) to measure tail length at docking and weaning and to determine the change in length between docking and weaning. Lambs were born in April and weaned at approximately 125 d of age. Within 24 h after birth, lambs were weighed and ear tagged, and rubber rings were applied to dock tails. Rings were applied just past the distal end of the caudal folds of the tail, which is just beyond where the folds attached to the tail. Time of rubber ring application was considered time of docking. Using a specially designed device, tail lengths were measured immediately after rubber ring application and at weaning. Lambs were weighed at weaning. Sex and breed type affected ($P < 0.005$) BW at docking and weaning; male and black-faced × white-faced lambs were heavier than female and white-faced × white-faced lambs. At docking, neither sex, breed type, nor the sex × breed-type interaction affected actual tail length or tail length adjusted for BW, although BW at docking was a significant ($P < 0.0001$) covariate. At weaning, the sex × breed type interaction affected ($P < 0.002$) actual tail length, which was greater ($P < 0.002$) for male, black-faced × white-faced lambs than for lambs of the other sex-breed-type classifications. At weaning, sex and sex × breed type affected ($P < 0.01$) tail length adjusted for the covariate BW at weaning. Breed type affected ($P < 0.006$) the change in actual tail length between docking and weaning (white-faced × white-faced, 2.4 cm, vs. black-faced × white-faced lambs, 2.7 cm). The data indicate clearly that tail length increased between docking and weaning.

Key words: Lambs, Tail Docking, Tail Growth

Introduction

Docking the tails of lambs is a traditional, but recommended, method for reducing the incidence of fly strike and its associated morbidity and mortality (Dykstra, 1942; Battaglia and Mayrose, 1981; Merck, 1986; Thomas et al., 2003; Lewis et al., 2010). Docking procedures have remained largely unchanged for at least 70 years, and a recommendation from that era was to amputate the tail approximately 5 cm to 8 cm distal to the union of the tail with the body of the lamb (Dykstra, 1942). Complete removal of the tail produces no discernable benefits for the sheep and can increase the incidence of rectal prolapse (for extensive discussion, see Thomas et al., 2003).

Because of concerns about rectal prolapse, the current recommendation in the United States is to dock tails at the distal end of the caudal folds, where the caudal folds attach to the tail (Battaglia and Mayrose, 1981; ASI, 2002; Lewis et al., 2010). The recommendation for tail docking in Australia and the United Kingdom is to retain sufficient tail "to cover the vulva in the case of female sheep and the anus in the case of male sheep" (DEFRA, 2000; PISC, 2006). In New Zealand, the recommendation is to leave the tail "long enough to cover the vulva in females and at a similar length in males" (MAF, 2005).

In discussing tail docking with sheep producers and students, the question has often arisen, "How much does a lamb's tail grow after docking?" Despite the interest in the length of docked tails, little information is available about the growth of a tail between docking and weaning. The only locatable article in a professional journal indicated that tail length usually increased between docking, weaning, and market age (Goodwin et al., 2007). Thus, the purpose of this study was to measure tail length at docking and weaning and determine tail growth between docking and weaning.

Materials and Methods

Animal Procedures

The United States Sheep Experiment Station Institutional Animal Care and Use Committee reviewed and approved all husbandry practices and experimental procedures used in this study.

Crossbred lambs (n = 229) were used for the study. The crosses were various combinations of Targhee, Rambouillet, Polypay, and Columbia breeds (i.e., white-faced × white-faced; n = 212; 99 ewes and 113 wethers) and Suffolk × white-faced (i.e., black-faced × white-faced; n = 17; 10 ewes, 6 rams, and 1 wether). The lambs were born between April 4 and April 11 of the same year and were weaned on August 12 or August 13 at 124.9 d ± 0.1 d of age. Unique, visual identification tags were inserted into each ear of each lamb within 24 h after birth. Lambs were weighed immediately before they were tagged and again at weaning. Ewe and lamb management was the same as that described in Leeds et al. (2012).

Rubber rings (Supervet Castrating Rings; Syrvet, Inc., Waukee, Iowa) were used to dock and castrate lambs that were assigned to the study. The rings were applied within 24 h after birth, immediately after lambs were tagged. In this study, the time when a rubber ring was applied to a tail was considered the time of docking, even though the act of applying a rubber ring to a tail is not, by definition, docking. For docking, the rings were applied just past the distal end of the caudal folds of the tail, which was just beyond where the caudal folds attached to the underside of the tail (Lewis et al., 2010). The portion of the tails, including the rubber rings, distal to the site of amputation typically dropped free within approximately 45 d.

Using a device and methods that have been described in detail (Goodwin et al., 2007), dock lengths were measured immediately after rubber rings were applied and again at weaning. The measuring device was graduated in 0.254-cm increments. At docking, the device was positioned firmly against the ischial tuberosities, and the distance to the cranial edge of the rubber ring was measured. At weaning, the device was positioned as described, and the distance to the free end of the docked tail was measured. A single, highly skilled technician applied rubber rings and performed all measurements.

Statistical Analyses

Methods in PROC GLM (SAS Inst. Inc., Cary, N.C.) were used to analyze the data. The models used in the analyses included terms for sex (i.e., female vs. male), breed type (i.e., white-faced × white-faced vs. black-faced × white-faced), and sex × breed type. When appropriate, BW was included in a model as a covariate. The dependent variables were tail length and BW at docking and weaning and tail growth between docking and weaning. Pair-wise contrasts (PDIFF = ALL option), with Tukey-Kramer methods for adjusting for multiple comparisons, were used to compare means.

Results

Table 1 contains the data from this study. Sex and breed type affected BW at docking and weaning; *P*-values ranged from < 0.005 to < 0.0001. At docking and weaning, respectively, male (5.4 kg and 44.6 kg) and black-faced × white-faced lambs (5.4 kg and 47.4 kg) were heavier than female (4.9 kg and 40.3 kg) and white-faced × white-faced lambs (4.8 kg and 37.4 kg).

At docking, neither sex, breed type, nor the sex × breed-type interaction affected actual tail length or tail length adjusted for BW, although BW at docking was a significant ($P < 0.0001$) covariate. At weaning, the sex × breed-type interaction affected ($P < 0.002$) actual tail length, which was greater ($P < 0.002$) for male, black-faced × white-faced lambs than for lambs of the other sex-breed-type classifications. At weaning, sex (female, 6.9 cm; male, 7.1 cm) and sex × breed type affected ($P < 0.01$) tail length, adjusted for the covariate BW at weaning. Breed type affected ($P < 0.006$) the change in actual tail length between docking and weaning: 2.4 cm for white-faced × white-faced and 2.7 cm for black-faced × white-faced lambs.

Using the parameter estimates derived from the statistical analyses of the data from this study, the following equations were constructed to predict tail length, in centimeters, at docking and weaning, respectively (SAS, 2009): 3.515 + (0.223 × birth weight, kg) and 5.943 + (0.028 × weaning weight, kg), where 3.515 and 5.943 are intercepts adjusted for breed type, sex, and sex × breed type, and 0.223 and 0.028 are covariates. The average difference between actual and predicted tail length at docking and weaning was −0.05 cm ± 0.03 cm and −0.02 cm ± 0.02 cm, respectively.

Table 1. Tail lengths and bodyweights of lambs when rubber rings were applied to dock tails and at weaning when lambs were 124.9 ± 0.1 d of age[1]

	White-faced × white-faced[2]		Black-faced × white-faced[3]		
Time of measurement	Female	Male	Female	Male	Pooled SE
			n		
	99	113	10	7	
			BW, kg		
Docking[4]	4.7	5.0	5.1	5.9	0.05
Weaning[5]	36.7	38.2	43.9	50.9	0.33
			Actual tail length, cm		
Docking	4.6	4.6	4.3	4.8	0.03
Weaning[6]	7.0[a]	7.0[a]	6.9[a]	7.6[b]	0.03
			Tail length adjusted for BW, cm[7]		
Docking[8]	4.6	4.3	4.5	4.5	0.03
Weaning[9]	7.0[a]	6.8[a,b]	7.0[a]	7.3[a,c]	0.02
			Docking to weaning actual tail growth, cm[10]		
	2.4	2.6	2.4	2.9	0.03

1 Rubber rings were applied within 24 h after birth.
2 White-faced crosses were various combinations of Targhee, Rambouillet, Polypay, and Columbia breeds.
3 Suffolk × white-faced crosses.
4 Sex ($P < 0.005$; female, 4.9 kg, vs. male, 5.4 kg) and breed type ($P < 0.003$; white-faced × white-faced, 4.8 kg, vs. black-faced × white-faced, 5.4 kg) were significant.
5 Sex ($P < 0.001$; female, 40.3 kg, vs. male, 44.6 kg) and breed type ($P < 0.0001$; white-faced × white-faced, 37.4 kg, vs. black-faced × white-faced, 47.4 kg) were significant.
6 Sex × breed was significant ($P < 0.002$). [a,b] Means with dissimilar superscripts differed ($P < 0.002$).
7 Least squares means.
8 The covariate BW at docking was significant ($P < 0.0001$).
9 Sex ($P < 0.01$; female, 6.9 cm, vs. male, 7.1 cm), sex × breed type ($P < 0.01$), and the covariate weaning weight ($P < 0.0001$) were significant. [a,b,c] For the sex × breed-type interaction, means with different superscripts differed ($P < 0.04$).
10 Breed type was significant ($P < 0.006$; white-faced × white-faced, 2.4 cm, vs. black-faced × white-faced, 2.7 cm).

Discussion

Between docking and weaning in the present study with male and female lambs, tail length increased an overall average of 2.6 cm ± 0.03 cm. Tail length increased for 100 percent of the lambs, and the increase ranged between 1.3 cm and 3.8 cm. As expected (Leeds et al., 2012), male and black-faced × white-faced lambs were heavier at docking and weaning than were female and white-faced × white-faced lambs. Body weight was a significant covariate for tail length. The equations using the covariates to adjust body weight at docking and weaning were reliable predictors of tail length, although additional research will be needed to determine whether the parameter estimates derived from the data in this study are useful for other subpopulations of crossbred lambs and other docking procedures.

In contrast to the data from this study, measured tail length in another study increased approximately 0.8 cm between docking (length 3.2 cm) and weaning (length 4.1 cm) and approximately 1.2 cm between docking and market (length 4.5 cm; Goodwin et al., 2007). The ages at weaning and market were not specified and BW data were not provided in that study (Goodwin et al., 2007). Furthermore, in Goodwin et al. (2007), tail length between docking and weaning and between docking and market decreased in 3.4 percent and 17.8 percent of the lambs, respectively, and the authors stated that the caudal folds were lost after docking. In Goodwin et al. (2007), a line was drawn on the ventral side of the tail at the distal end of at least one caudal fold to mark the site of docking. The authors stated that, "If one caudal fold was shorter than the other, the line was drawn on the shorter of the two and the procedure was carried out at this location." Perhaps this docking procedure resulted in the loss of the caudal folds.

The average actual increase in tail length in the present study was approximately twice that reported previously (Goodwin et al., 2007), and caudal folds were clearly intact at docking and weaning in the present study. Based on Thomas et al. (2003), ensuring that the caudal folds remain intact after docking may reduce fecal contamination of wool and further reduce the chances of fly strike. In addition, tail length at docking in the present study was similar to tail length at market in Goodwin et al. (2007). The United States Sheep Experiment Station tail-docking procedure seems to leave tails somewhat longer than the United States "norm," assuming tails are docked near the recommended anatomical location, but somewhat shorter than the length recommended in Australia, New Zealand, and the United Kingdom (DEFRA, 2000; Goodwin et al., 2007; MAF, 2005, Thomas et al., 2003; PISC, 2006). Even though the present study and the study of Goodwin et al. (2007) do not seem equivalent, both studies indicate clearly that tail length should be expected to increase after docking.

Conclusions

Tail length consistently increased between docking and weaning. The tail-docking procedure used in this study left the caudal folds intact, which is considered to be beneficial to the well-being of sheep.

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Accuracy of Ultrasonographic Diagnosis of Sex and Effect of Sex and Birth Type on Biparietal Diameter of Saanen Goat Fetuses

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Summary

The effects of sex and birth type on biparietal diameter (BPD) were examined from 6th to 14th weeks of gestation in 29 pregnant Saanen does by ultrasonography and after birth by observing the kids directly. Fifteen does delivered singles, 13 had twins and one goat had quadruplet males. Twelve of the twins were male and 13 were female; 7 singles were male and 8 were female. In twin pregnancies, the most accurate period for diagnosis of sex of the fetus by observation of the position of the genital tubercle was the 9th week of gestation. However in singles, two errors (13 percent) were made at the 9th week. Two-way anova analysis revealed that birth type did not affect BPD of Saanen goat fetuses, and sexes did not differ until the 14th week ($P < 0.05$). Even then, the difference was too small to be useful to predict the sex of the offspring. Chi-square test was applied to compare the success rates of ultrasonography for prediction of fetal sex in different weeks of gestation. Ratio for success of sex determination by ultrasonography was greater from the 9th week of gestation compared with earlier periods in twin pregnancies ($P < 0.001$). On the other hand, there were no significant differences among gestation weeks in terms of ratio for success of sex determination by ultrasonography in single pregnancies ($P > 0.05$). Thus it is concluded that sex of the fetus can be diagnosed directly at the 9th week, but one cannot establish the sex of the fetuses by using only ultrasonographic measurements of BPD in either twin or single pregnancies in Saanen goats.

Keywords: Biparietal Diameter, Birth Type, Saanen Fetal Goat Sex, Ultrasonography

Introduction

Ultrasonography is an important non-invasive technique for fetal-sex assessment. Early fetal sexing provides the best planning for the acquisition and commercialization of animals, and allows the marketing of male and female fetuses, while still in utero and the differentiation of fetuses for use in meat or milk herds (Santos et al., 2007b). In cows, early sex determination permits identification of male and female co-twins, which could lead to a female freemartin. Fetal sex determination by ultrasonography was performed previously in cattle, buffaloes, horses, sheep and goats (Mari et al., 2002; Tainturier et al., 2004; Santos et al., 2007a, b; Ali and Fahmy 2008; Azevedo et al., 2009; Moraes et al., 2009).

The genitalia of the fetus develop from the mesenchyme of the ventral abdominal wall, between the tail, the hindlimbs and the umbilical cord (Yotov et al., 2011). Penis in males and clitoris in females differentiate from the genital tubercle (GT), which is an embryonic structure. Initially, the location of the GT is between the hind limbs, and the sex cannot be identified at this point. The GT later migrates towards the umbilical cord and appears as hyperechogenic points in males, whereas it migrates towards the tail and appears as echogenic genital swellings in females (Mari et al., 2002; Yotov et al., 2011).

Amer (2010) reported evaluation of fetal sex of both single and multiple fetuses in goats during the stages of 40 d to 60 d, 61 d to 70 d and 90 d to 109 d after mating. Optimum results were achieved in the first stage, and the accuracy of sex diagnosis varied among these stages, being 93 percent, 82 percent and 58 percent, respectively.

In goats, Azevedo et al. (2009) reported the presence of a bilobar hyperechoic structure near the umbilical cord that allowed correct diagnosis as a male fetus. When this structure was not observed, the fetus was diagnosed as female at the same period of pregnancy.

Ultrasonography was used in horses and cattle to identify fetal sex by visualizing the penis or scrotum or the location of the genital tubercle (Mari et al., 2002). Fetal sex was determined by visualizing the external genitalia (penis, prepuce, scrotal bag, nipples, and genital swelling) and/or according to the location of the GT. In several studies, the sex of the fetuses was confirmed by visualizing the external genitalia after birth (Santos et al., 2006, 2007a, b; Amer 2008, 2010; Azevedo et al,. 2009; Moraes et al., 2009).

Biparietal diameter (BPD) involves the measuring of the skull of the fetuses when it has achieved an oval shape, and the flax cerebri mid-line is dividing the hemispheres into two equal parts. The aim of this study was to detect effects of sex and birth type on BPD during different stages of gestation in Saanen goat fetuses.

Materials and Methods

Animals and management

Twenty nine pregnant Saanen does aged between 1-½ years and 3 years, kept under standard management conditions, were used for this study. The goats were observed carefully for estrus twice daily, morning and afternoon, and were mated by the buck naturally. The day of mating was designated as day 0 of gestation.

Experimental design

Fifteen of the does were examined transrectally once a week, on the same weekday, from the 6th through 14th weeks of gestation. Another 14 does were examined transrectally only until the 10th week of gestation and then transabdominally until the 14th week of gestation. Ultrasonography was performed with a linear transducer (5.0 mHz to 8.0 mHz) using a B-mode, real-time scanner (Medison SA 600V, South Korea) adapted to a PVC in order to facilitate the manipulation into the animal's rectum. Before transrectal examination, the rectum was evacuated manually and the ultrasound probe was covered by a coupling gel and introduced into the rectum after evacuation while does were standing. For transabdominal ultrasonography the goats were scanned in lateral recumbency or in the standing position, after shaving the ventral abdominal wall beside the udder. In order to detect the genital tubercle easily, the main parts of the fetus were established first.

After visualizing the fetus at the early periods of gestation, sex of the fetuses was identified according to the location of the GT (Fig. 1a). In later periods of gestation, the sex was identified by visualizing the penis, prepuce, scrotal bag (Fig. 1b), nipples, or vulva (Fig. 1c). Images were frozen on the screen and measured with built-in electronic callipers. The BPD was measured when the skull of the fetuses was visualized oval in shape, with the flax cerebri mid-line dividing the hemispheres into two equal parts. The measurements were taken from the outer surface of the proximal calvarium to the inner surface of the distal calvarium (Fig. 1d).

During the last week of ultrasonography, the estimation of the skull became more challenging. The aim was to establish the fetal sex and BPD, so the ultrasound examinations lasted as long as was necessary to obtain optimal scanning conditions, which always took a maximum of 15 minutes.

Statistical analysis

For statistical analysis, the two-way anova method was used to test for effects of sex and birth type on BPD at each week of gestation (6 weeks through 14

Figure Legends: Fig. 1a. Male fetus; genital tubercle is close to the umbilical cord (arrow).
Fig. 1b. Fetal scrotal bag at the 10th week of the gestation (arrows).
Fig. 1c. Vulvar region in a female fetus (arrow).
Fig. 1d. Biparietal diameter of a fetus.

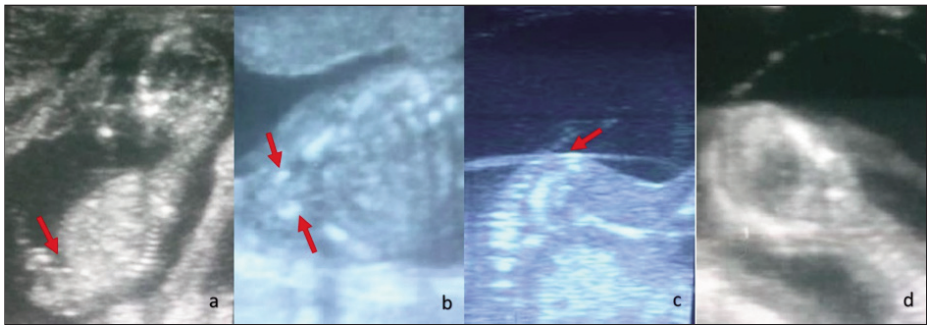

Table 1. Mean biparietal diameters (mm) and and standard errors (SE) from the 6th week to the 14th week of gestation and after birth in relation to sex of the fetuses in singleton and twin Saanen goat fetuses.

Gestation Period	Sex				Birth Type				Significance	
	Male		Female		Single		Twin			Birth
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Sex	Type
6. Week	12.3	0.4	11.8	0.4	12.0	0.5	12.1	0.3	NS	NS
7. Week	16.1	0.5	15.9	0.5	16.0	0.6	16.0	0.4	NS	NS
8. Week	20.2	0.7	19.1	0.7	19.2	0.8	19.7	0.6	NS	NS
9. Week	24.6	0.7	23.5	0.8	24.3	0.8	23.8	0.6	NS	NS
10. Week	29.7	0.8	28.7	0.9	29.5	1.0	29.0	0.7	NS	NS
11. Week	34.2	0.6	33.2	0.7	34.1	0.8	33.3	0. 6	NS	NS
12. Week	37.3	0.6	36.5	0.6	37.7	0.7	36.1	0.5	NS	NS
13. Week	41.5	0.6	40.3	0.6	41.1	0.7	40.7	0.5	NS	NS
14. Week	45.7	0.4	44.2	0.4	4.5	0.5	44.9	0.4	*	NS
After Birth	83.8	1.0	80.8	1.0	82.8	1.1	81.8	0.9	*	NS

NS: Not significant ($P > 0.05$)
*: ($P < 0.05$)

weeks) in Saanen goat fetuses. Chi-square test was applied to compare the success rates of ultrasonography for prediction of fetal sex at different weeks of gestation.

Results

The results of weekly ultrasound examinations were compared with either identified sex or BPD of the offspring at birth. Of 45 fetuses examined in 29 Sannen does, 44 were born and one was mummified. Fifteen does delivered singles, 13 had twins and one had quadruplets. Twelve of the twins were male and 13 were female (Appendix Table 1, page 14). Seven of the singles were male and eight were female (Appendix Table 2, page 15). One goat gave birth to four males, but during ultrasound examinations only two of the fetuses could be distinguished and these two were evaluated as male (Fig. 1c). Transabdominal ultrasonography was found to be more difficult than transrectal ultrasonography and required more time to examine the fetuses in order to distinguish either BPD or fetal sex.

The mean weekly measurements of BPD and the sex of the fetuses during different stages of gestation are given in Table 1. After birth, the fetal sex was confirmed for each kid. Fetal sex of two of the offspring was evaluated incorrectly; the others were established correctly.

Statistical analysis

According to the two-way anova analyses, the BPD differed between the sexes only at the last (14th) week and after birth ($P < 0.05$). The BPD did not differ with birth type at any stage. Ratio for success of sex determination by ultrasonography was greater from the 9th week of gestation onward compared with earlier periods in twin pregnancies ($P < 0.001$). On the other hand, there were no significant differences between gestation weeks in terms of ratio for success of sex determination by ultrasonography in single pregnancies ($P > 0.05$) (Table 2). In the 15 single pregnancies in this study, two false diagnoses were obtained (13 percent). In one doe, the false diagnosis may be attributed to the ultrasonographic presentation of the fetus at the time of the examination. In this doe, the fetus was recorded as female till the 9th week, then it was recorded as male, but was female at birth, so diagnosis after 9th week was false. In the second doe, from the onset of examination till the end the fetus was assumed as male, but at birth it was distinguished as female. In examination of this fetus, the sex indicators or their locations were unclear or absent. Conversely, results were changed in 5 of 13 does (38 percent) in 9th week in twin pregnancies. When compared with afterbirth results, they were subsequently detected correctly through the end of the study. The two false results in single pregnancies may have occurred because there was not as much extra care as was

Table 2: Successful prediction rates (%) for sex of the fetus according to the weeks of ultrasonographic examinations.

Weeks	Single (%)	Twins (%)	All of the data set (%)
6	61.5	80[b]	73.7[c]
7	60.0	84[b]	75[c]
8	73.3	80[b]	77.5[bc]
9	66.7	100[a]	87.5[abc]
10	85.7	100[a]	94.6[a]
11	85.7	95.7[ab]	91.6[ab]
12	86.7	100.0[a]	95[a]
13	86.7	100[a]	94.9[a]
14	86.7	100[a]	95[a]
Chi-square	**8.822 (NS)**	**26.14 (***)**	**23.855 (**)**

NS: Not significant ($P>0.05$).
[a, b, c]: Differences between the means lacking a common letter in the same column are significant (**= $P<0.01$; ***= $P<0.001$)

taken during ultrasonographic examinations in twin pregnancies.

In the present study, accuracy of ultrasonographic examinations was greatest at the 9th week of gestation, which is in line with recommendations by Santos et al. (2007b). The most frequent variations were observed in the diagnosed sex of the fetuses in Saanen goats after day 63 (9th week) of pregnancy. Oliveira et al. (2005) reported that GT migration took place around 48.9 d ± 1.8 d in Saanen goats. As reported by Santos et al. (2007b), the GT migration time affects the accuracy of fetal sexing in small ruminants. They examined migration of the genital tubercle between the days of gestation 40 d to 60 d with ultrasonography, 55 d to 70 d transrectally, and 100 d to 120 d transabdominally. They found that migration of GT occurred around day 50 of the pregnancy in goat fetuses and suggested that examinations should take place between 55 d to 70 d of pregnancy to avoid false diagnosis of the sex of the fetuses. Santos et al. (2007a) recommended multiple examinations for fetal sexing in triplet pregnancies, as more fetuses increased the risk of failures, which agrees with our results. In the present study, we could not visualise the sex of two fetuses nor even their presence in one quadruplet pregnancy.

It was very difficult to determine the sex of the fetus with an examination lasting less than 15 minutes at only one plane. It has been reported that the appearance of the genital tubercle may change (Yotov et al., 2011). In the latter study, 6 percent of fetuses had unidentified sex in buffaloes; these false diagnoses could be attributed to the absence or unclear visualisation of the sex indicators and their locations. In the present study, diagnosis of the sex of some fetuses was altered as the appearance of this structure changed after their sex was established initially. Yotov et al. (2011) reported that the sex can be determined best in sagittal or cross-sectional position, whereas Azevedo et al. (2009) considered the longitudinal plane best in ovine or caprine fetuses.

In this study, there were more undetermined cases of fetal sex at weeks 11, 13 and 14 due to the growth of the fetuses. Gonzalez de Bulnes et al. (1998) reported that in Manchega dairy ewes the estimation of fetal development using a transrectal ultrasound device can be efficient to detect pregnancy until day 90 to 91, after which the fetus cannot be detected properly with this technique. Similarly, Yotov et al. (2011) observed that after the fetus was grown, accuracy of sex determination could be reduced in buffaloes. Pedreira et al. (2001) reported a variation in the orientation of the genital tubercle during their study period in human fetuses. In the present study, comparison of the transrectal and transabdominal approaches during the 10th through 14th weeks of gestation indicated that the transabdominal technique was more difficult and required more time to evaluate and establish the sex and the BPD.

Biparietal diameter has been used to predict fetal sex in human beings (Mazza et al., 1999). These authors found that, for accurate detection of fetal sex in humans, the threshold above 23 mm of BPD provided the best results. Reichle and Haibel (1991) reported that the fetal BPD measurement was most accurate in mid-gestation due to the larger size of the fetus; this study's results agree with theirs. Abdelghafar et al. (2011) measured weekly BPD of the fetuses starting from 6th week until the end of gestation in Saanen goats. In this study, ultrasonographic examinations were not continued beyond week 14, due to the difficulties of imaging the fetuses as fetal size increased.

In one animal, a mummified fetus was evaluated after birth. The animal's pregnancy was normal during our ultrasonographic examinations, so the fetus apparently died after the completion of ultrasonographic examinations, i.e., after the 14th week of gestation.

Conclusions

In conclusion, one cannot establish the sex of the fetuses by using only ultrasonographic measurements of BPD in either twin or single pregnancies in Saanen does. Birth type did not affect BPD of Saanen goat fetuses, and sexes did not differ until the 14th week of gestation. Even then, the difference was too small to be useful to predict the sex of the offspring. Direct observation of the genital tubercle appears to be necessary to evaluate fetal sex, and that is difficult in multiple pregnancies.

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Appendix Table 1. Measurements of biparietal diameter (mm) and predicted sex of twin fetuses from 6th week to 14 week of gestation and after birth in Saanen goats.

		Gestation periods									
Groups	Doe id.	6. week BPD (mm)	7. week BPD (mm)	8. week BPD (mm)	9. week BPD (mm)	10. week BPD (mm)	11. week BPD (mm)	12. week BPD (mm)	13. week BPD (mm)	14. week BPD (mm)	After birth BPD (mm)
Female/Male	1	10.1 (M) 11.2 (M)	21.5 (F) 22.2 (M)	26.6 (M) 28.0 (M)	30.6 (F) 31.0 (M)	31.2 (F) 32.4 (M)	33.1 (F) 34.0 (M)	35.3 (F) 36.0 (M)	38.0 (F) 38.6 (M)	43.0 (F) 44.3 (M)	80.0 (F) 80.4 (M)
	2	8.94 (M) 8.92 (F)	11.7 (M) 10.8 (F)	17.3 (F) 16.8 (F)	21.2 (M) 20.5 (F)	28.7 (M) 27.6 (F)	33.0 (M) 32.1 (F)	35.4 (M) 34.2 (F)	39.2 (M) 38.5 (F)	47.0 (M) 46.3 (F)	85.0 (M) 82.0 (F)
	3	13.0 (M) 12.8 (F)	13.9 (M) 13.4 (F)	23.3 (M) 23.0 (F)	27.8 (M) 26.0 (F)	34.0 (M) 32.8 (F)	36.0 (M) 35.2 (F)	38.4 (M) 36.7 (F)	42.0 (M) 39.2 (F)	46.3 (M) 43.2 (F)	86.0 (M) 80.0 (F)
	5	11.2 (M) 12.2 (M)	16.2 (M) 17.3 (M)	18.3 (M) 19.0 (M)	22.8 (F) 24.2 (M)	25.4 (F) 27.0 (M)	31.3 (F) 33.0 (M)	33.0 (F) 34.2 (M)	36.0 (NI) 38.2 (M)	44.5 (F) 45.6 (M)	85.0 (F) 87.0 (M)
	6	13.0 (F) 12.3 (M)	16.2 (F) 15.3 (M)	18.3 (F) 17.6 (M)	22.0 (F) 21.3 (M)	NI 26.0 (M)	27.3 (F) 29.0 (NI)	33.3 (F) 32.0 (M)	42.4 (F) 43.0 (M)	47.3 (F) 46.0 (M)	85.0 (F) 80.0 (M)
	7	12.8 (M) 13.2 (M)	17 (F) 16 (M)	21.4 (F) 22.3 (M)	28.2 (F) 29.1 (M)	34.6 (F) 35 (M)	38 (M) 38.2 (M)	38.7 (F) 38.9 (M)	41.2 (F) 42 (M)	43.0 (F) 44.0 (M)	80.0 (F) Mummified
	13	11.2 (F) 13.4 (M)	15.8 (F) 16.7 (M)	18.0 (F) 21.0 (M)	22.0 (F) 23.4 (M)	24.4 (F) 25.6 (M)	32.8 (F) 33.2 (M)	37.6 (F) 38.0 (M)	44.6 (F) 44.7 (M)	45.6 (F) 46.2 (M)	78.0 (F) 80.0 (M)
Only Female	9	13.1 (F) 14.0 (F)	16.0 (F) 15.8 (F)	19.0 (F) 18.8 (F)	22.0 (F) 21.2 (F)	28.2 (F) 26.0 (F)	33.5 (F) 32.0 (F)	37.8 (F) 36.7 (F)	41.2 (F) 40.9 (F)	44.0 (F) 42.7 (F)	82.0 (F) 80.0 (F)
	10	14.5 (F) 13.2 (F)	16.3 (F) 15.3 (F)	18.3 (F) 17.6 (F)	23.0 (F) 23.2 (F)	29.9 (F) 29.8 (F)	37.5 (F) 36.5 (F)	38.3 (F) 38.4 (F)	39.4 (F) 39.6 (F)	43.3 (F) 44.0 (F)	82.0 (F) 83.0 (F)
	12	13.1 (F) 13.6 (M)	16.3 (F) 17.2 (M)	22.6 (M) 18.4 (F)	26.0 (F) 24.3 (F)	30.8 (F) 31.2 (F)	33.2 (F) 34.1 (F)	36.5 (F) 37.0 (F)	42.7 (F) 42.7 (F)	45.2 (F) 45.3 (F)	84.0 (F) 85.0 (F)
Only Male	4	10.4 (F) 9.0 (M)	14.5 (F) 13.0 (M)	17.3 (F) 16.7 (M)	22.0 (M) 19.0 (M)	NI 25.3 (M)	NI (M) 29.0 (M)	29.6 (M) 31.0 (M)	38.3 (M) 36.0 (M)	46.2 (M) 43.0 (M)	80.0 (M) 75.0 (M)
	8	12.8 (M) 11.7 (M)	17 (M) 16.7 (M)	18.3 (M) 18.2 (M)	21.4 (M) 20.9 (M)	28.7 (M) 27.4 (M)	32.4 (M) 31.8 (M)	38.3 (M) 37.9 (M)	42.8 (M) 41.7 (M)	46.0 (M) 45.7 (M)	83.0 (M) 80.0 (M)
	11	12.7 (M) 13.2 (M)	16.6 (M) 17.0 (M)	18.0 (M) 18.3 (M)	22.8 (M) 23.0 (M)	26.5 (M) 26.8 (M)	33.3 (M) 34.0 (M)	37.4 (M) 38.2 (M)	42.3 (M) 43.5 (M)	44.8 (M) 45.4 (M)	80.0 (M) 82.0 (M)

NI: Not Identified

Appendix Table 2. Measurements of biparietal diameter (mm) and predicted sex of single fetuses from 6th week to 14 week of gestation and after birth in Saanen goats.

	Gestation periods									
No	6. week BPD (mm)	7. week BPD (mm)	8. week BPD (mm)	9. week BPD (mm)	10. week BPD (mm)	11. week BPD (mm)	12. week BPD (mm)	13. week BPD (mm)	14. week BPD (mm)	After birth BPD (mm)
14	12.8 (F)	17.7 (F)	28.2 (M)	31.0 (M)	32.8 (M)	35.2 (M)	38.2 (M)	42.0 (M)	47.0 (M)	90.0 (M)
15	9.6 (F)	17.1 (F)	20.4 (F)	23.2 (F)	26.3 (F)	28.0 (F)	33.0 (F)	41.6 (F)	44.0 (F)	80.0 (F)
16	10.8 (M)	14.6 (M)	18.4 (M)	23.0 (M)	33.0 (M)	35.5 (M)	39.0 (M)	41.8 (M)	45.0 (M)	80.0 (F)*
17	10.2 (F)	14.4 (F)	15.8 (F)	23.1 (F)	28.4 (F)	31.0 (F)	36.6 (F)	38.0 (F)	46.3 (F)	80.0 (F)
18	11.1 (F)	15.4 (F)	18.0 (F)	24.2 (M)	NI	33.6 (M)	36.0 (M)	38.2 (M)	46.8 (M)	90.0 (F)*
19	11.1 (NI)	14.3 (M)	16.4 (M)	21.0 (F)	27.6 (F)	33.0 (F)	35.6 (F)	37.0 (F)	41.3 (F)	72.0 (F)
20	8.3 (M)	12.2 (F)	14.7 (M)	21.8 (F)	33.2 (M)	36.0 (M)	38.4 (M)	43.1 (M)	46.2 (M)	85.0 (M)
21	11.4 (M)	16.3 (M)	18.2 (M)	23.0 (M)	28.0 (M)	NI (F)	32.6 (F)	34.0 (F)	36.0 (F)	69.0 (F)
22	16.1 (F)	18.2 (F)	24.3 (F)	30.4 (F)	38.7 (M)	40.2 (M)	43.9 (M)	44.2 (M)	46.3 (M)	89.0 (M)
23	14.2 (F)	17.0 (F)	19.6 (F)	23.0 (F)	28.5 (F)	36.2 (F)	41.4 (F)	42.8 (F)	44.2 (F)	80.0 (F)
24	12.2 (NI)	16.3 (M)	18.4 (M)	23.0 (M)	26.5 (M)	31.4 (M)	36.2 (M)	38.6 (M)	47.2 (M)	85.0 (M)
25	12.7 (F)	15.7 (M)	19.4 (M)	23.0 (M)	26.7 (M)	34.3 (M)	38.3 (M)	43.0 (M)	47.5 (M)	93.0 (M)
26	11.2 (F)	15.8 (F)	18.0 (F)	22.0 (F)	24.4 (F)	32.8 (F)	37.6 (F)	44.6 (F)	45.6 (F)	78.0 (F)
27	15.4 (M)	18.3 (M)	23.8 (M)	31.0 (M)	35.6 (M)	38.8 (M)	42.3 (M)	44.4 (M)	46.2 (M)	89.0 (M)
28	13.4 (M)	16.7 (M)	21.0 (M)	23.4 (M)	25.6 (M)	33.2 (M)	38.0 (M)	44.7 (M)	46.2 (M)	85.0 (M)

NI: Not Identified
*: Misdiagnosed Cases

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Growth and Performance of Meat Goat Kids from Two Seasons of Birth in Kentucky.[1,2]

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Summary

Little information is available on the impact of season of kidding on growth and performance of meat-goat kids. However, seasonal market trends have many producers in the southeastern United States kidding in the late fall and winter, when animals must be supplemented to meet nutritional needs. Because of this, a study was designed with the objectives being to evaluate the effect of season of birth and other factors on kid survival to weaning and performance from birth to weaning in meat-goat kids. One hundred and twenty commercial-meat-type does were used in this study. The does were bred for kidding either in the fall (October, November, and December) or spring (March, April, and May) seasons. Data collected included birth weight, birth type, sex, 60 d weight, and 90 d weight. Season of birth had a significant effect on birth ($P < 0.0001$) and 90 d wt ($P = 0.0063$), and ADG between 60 d and 90 d ($P = 0.0003$), with fall-born kids being heavier and having higher daily gains. The interaction between year and birth type was significant ($P = 0.0004$) for birth weight and the sex by birth/rearing type interaction was significant for 60-d wt ($P = 0.0003$) and ADG to 60 d ($P = 0.0002$). These data indicate that season of birth has an impact on some performance traits in meat goat kids. These differences can impact profitability and need to be studied in more detail to determine specific impacts on productivity and profitability of the meat goat industry.

Key Words: Meat Goat, Season of Birth, Kid Performance, Preweaning Growth.

Introduction

Meat goat markets have shown seasonal price differences with higher prices in the spring when supply is generally low and demand is high due to several ethnic holidays. Many producers are breeding to target the peaks in price that occur around ethnic holidays (Coffey, 2002). To do this, many producers have started breeding does to kid in the fall and winter months. However, there is little information available to determine if season of birth impacts growth and survival traits of meat-goat kids. In fact, Shrestha and Fahmy (2007) stated that scientific knowledge of meat-goat production is negligible compared to other livestock and poultry species. Research with cattle and sheep has shown differences in weaning weight for different calving and lambing seasons (McCarter et al. 1991; Gaertner et al. 1992; Lewis et al. 1996; and Casas et al., 2004). Previous studies with sheep and goats indicate that there are seasonal differences in survival rate of young stock (Awemu et al., 1999; Hailu et al., 2006; Husain et al., 1995; Shelton and Willingham, 2002). Because of findings in other species and limited information in goats, a project was designed to determine the effect of fall or spring kidding seasons on 1) birth, 60-d, and 90-d wt; 2) growth rate from birth to 90 d; and 3) survival from birth to 90 d in Boer-cross meat goats.

Materials and Methods

A total of 543 commercial, meat-goat kids born over three years was available for this project. The kids were produced in the herd at Kentucky State University in Frankfort, Kentucky; Latitude: 8.12, Longitude: 84.88, elevation: 228.14 m. Kids were born in the fall (October – December) or spring (March – May) of each year. All kids were the result of natural service and received similar nutrition and management while nursing.

Does were assigned randomly to breeding groups to produce kids in either the fall or spring and remained in the assigned group throughout the study. All does were exposed by natural service for a 60-day breeding season with target-kidding dates of October 15 to December 15 for the fall-kidding season, and March 15 to May 15 for the spring-kidding season. Data collection ended with the spring 2008 kidding season. More information on the management and breeding can be found in Andries (2011).

The does were maintained on tall fescue *(Lolium arundinaceum)* pastures during the year with a small amount of native, warm-season grass available during the summer. Does were fed tall fescue hay during the winter and when forage was limited. Forage analyses were conducted on the hay used each year (Table 1), and does were supplemented to meet the NRC (2007) requirements for late gestation and lactation using a commercial 15 percent CP, 75 percent TDN pellet from Bagdad Roller Mills, Bagdad Ky. (Table 1). Additional doe management was conducted as described by Andries (2011).

Kids were born on pasture and then moved, with their dam, to individual kidding stalls for 48 hours. Kids were given an ear tag, injected with selenium (1/2 cc of BoSe), and navels were treated with 7 percent iodine within 12 h of birth. Sex of the kid and birth type were recorded within 12 h of birth. After the 48 h, the doe and kid(s) were moved to a new pasture. A survival code was used to determine which kids were born dead; died between birth and 60 d; died between 60 d and weaning; or were weaned. Kids were not treated for internal parasites until after weaning and buck kids were not castrated. All kids were vaccinated for *Clostridium perfringens* type C&D and tetanus before weaning.

Kids were weaned at an average age of 90 d. Kid weights were taken at birth, 60 d, and 90 d. Both spring- and fall-born kids were creep fed, ad lib, between e60 d and 90 d on the same pellet feed used to supplement the does (Table 1). Kids had access to pasture and hay beginning 48 h after birth. The fall-born kids were on dormant fescue pastures, while the spring-born kids had access to fresh fescue pastures after being moved from the kidding pens. Average daily gain was calculated for birth to 60 d and 90 d and between 60 d and 90 d. At weaning, body condition score, using the five-point system for goats, and FAMACHA© score were recorded for each kid using the same trained individual at all collection times.

A total of three sires was used in each breeding season, and seven sires were used during the project. All sires were used in both spring- and fall-kidding seasons and represented in more than one year of the project. Sires used were registered Boer bucks and were listed as purebred or full blood on their papers. Sires were replaced only as needed due to death or physical injury. Breeding for the first fall-kid crop was done as a group breeding with three bucks; all other mating was done using single-sire breeding pastures.

Data were analyzed using Proc Mixed (SAS Institute Inc. Cary N.C.). Season, birth type/rearing type, sex, and project year were included as fixed effects. All possible two- and three-way interactions were tested. Non-significant interactions were removed for final analysis. Day of birth within kidding season was used as a covariate for birth weight, and age was included for 60 d and 90 d wt.

Table 1. Nutritional composition of hay and supplement.[ab]

Feed	DM	CP	ADF	NDF	TDN	NEm	NEg
Supplement	89.78	16.73	21.52	40.72	75.47	0.84	0.56
2005 Hay	90.5	7.1	47.6	73.2	44.2	0.35	0.10
2006 Hay	89.8	8.7	4.3	75.1	41.6	0.3	0.06
2007 Hay	88.6	8.6	44.5	71.5	43.9	0.34	0.10

[a] Analysis for supplement provided by Bagdad Roller Mills, Bagdad, Ky; hay test conducted at the Kentucky Department of Agriculture Forage Quality Lab, Frankfort, Ky.

[b] DM = % Dry Matter, CP = % Crude Protein, ADF = % Acid Deterigent Fiber, NDF = % Neutral Detergent Fiber; TDN = % Total Digestability Nutrients, NEm = Net Energy for Maintaince in Mcal/lb, NEg = Net Energy for Gain in Mcal/lb. All values are reported on a DM basis.

Table 2: Least square means and standard errors for season of birth and sex of kid.[a]

Trait[b]	Spring	Fall	P value Season[c]	Male	Female	P value Sex[c]
Birth wt	3.37 ± 0.04	3.68 ± 0.05	<0.0001	3.72 ± 0.04	3.34 ± 0.04	< 0.0001
60-d wt	13.6 ± 0.24	13.5 ± 0.27	0.7598	14.8 ± 0.31	12.3 ± 0.30	< 0.0001
ADG 60 d	0.16 ± 0.003	0.15 ± 0.004	0.0809	0.17 ± 0.004	0.14 ± 0.004	< 0.0001
90-d wt	16.9 ± 0.34	18.1 ± 0.38	0.0063	18.6 ± 0.34	16.4 ± 0.34	< 0.0001
ADG 60 – 90 d	0.13 ± 0.01	0.17 ± 0.01	0.0003	0.16 ± 0.01[y]	0.13 ± 0.01[z]	0.0019
ADG to 90 d	0.15 ± 0.003	0.16 ± 0.004	0.0494	0.16 ± 0.003	0.14 ±0.003	< 0.0001
BCS	2.8 ± 0.04	2.4 ± 0.04	<0.0001	2.6 ± 0.04	2.6 ±0.04	0.5292
FAMACHA©	2.5 ± 0.08	2.4 ± 0.08	0.2150	2.5 ± 0.07	2.5 ± 2.07	0.7813

[a] Data are expressed as value ± standard error.
[b] Birth, 60-d, and 90-d wts are measured in kg, ADG is in kg/d, BCS = body condition score and is on a 1 to 5 scale, FAMACHA© is on a 1 to 5 scale with 1 being non-anemic and 5 very anemic.
[c] P value for comparison of means of either season or sex of kid.

Results and Discussion

The data set contained 543 (286 spring and 257 fall) kidding records for analysis. The average birth date for spring-born kids was March 30 and for fall-born kids was November 18. Least square means for the different traits are presented in Table 2.

Birth Weight:

Birth weight was collected within 12 h of birth for all kids born, including stillborn kids. The data set included 541 birth observations used for the analysis and indicated that birth weight was significantly *(P < 0.001)* affected by season of birth, sex of kid, birth type, and year by birth-type interaction. Spring-born kids were significantly lighter than fall-born kids (3.37 kg vs 3.68 kg, respectively). Other factors that affected birth weight (Table 2) included sex *(P < 0.0001)*, date of birth *(P = 0.0021)*, and birth type *(P < 0.0001)*. These effects were expected and are generally accepted as factors that influence birth weight. The interaction between birth type and birth year was also significant *(P = 0.0004)*. Within a year, the differences between birth types varied but the overall ranking did not change (Table 3). The direct effect of project year did not affect birth weight.

Previous work on the effects of season of birth on kid performance has primarily focused on differences between wet and dry seasons. The wet season increased birth weight in kids when compared to the dry season according to reports by Baiden (2007) and Awemu et al. (1999). However, Al-Shorepy et al. (2002) reported that birth weight was not affected by season. Previous reports indicated that sex of kid is significant for birth weight, similar to our study (Al-Shorepy et al., 2002, Browning and Leite-Browning, 2011; Mourad and Anous, 1998; Wilson and Light, 1986). Other researchers have found that type of birth has an impact on birth weight similar to this project (Browning and Leite-Browning, 2011; Martiney et al., 2010; Mellado et al., 2011; Mourad and Anous, 1998; Sanchez et al., 1994). However, Baiden (2007) reported no difference between single- and twin-born kids but showed that single-born were heavier than triplet-born kids.

60 d Weight

Weight at 60 d was significantly affected by sex *(P < 0.0001)*, rearing type *(P < 0.0001)*, and the interaction between sex and rearing type *(P = 0.0003)*. Season of birth did not influence 60 d weight. Buck kids were heavier than doe kids, similar to birth weight. Least square means for kids based on birth and rearing type are listed in Table 4.

Average daily gain between birth and 60 d was significantly *(P < 0.0001)* affected by rearing type and sex of kid. Spring-born kids tended to have higher ADG between birth and 60 d *(P = 0.0809)* than fall-born kids. Least square means for ADG from birth to 60 days of age for season of birth and sex of kid are listed in Table 2.

Mourad and Anaus (1998) reported that male kids were heavier at all weights after birth and single-born kids had faster growth rates between birth and 30 d. Ndlovu and Simela (1996) reported that kids born in the dry season were heavier at 60 d but sex did not impact growth to 60 d. Differences among these studies may be due to climate differences between Kentucky and the regions where these studies were conducted.

Table 3. Least square means and standard errors for birth weight; interaction between birth type and year of birth.[a]

Birth Type	Year 1	Year 2	Year 3
Single	4.14±0.18[bz]	3.73±0.13[bz]	3.87±0.13[bz]
Twin	3.55±0.05[cx]	3.34±0.06[cy]	3.52±0.06[cx]
Triplet	2.89±0.09[dx]	3.27±0.11[dy]	3.44±0.10[cz]

[a] Values are listed as birth weight in kg ± standard error for each birth type within each year of the project.
[bcd] Values within a column with different superscripts differ significantly (*P<0.01*).
[xyz] Values within a row with different superscripts differ significantly (*P<0.01*).

Table 4. Least square means and standard errors for growth traits by type of birth and rearing.[a]

Trait[b]	SN – SN	TW – SN	TW – TW	TR – SN	TR – TW	TR – TR
N for 60d	53	45	250	14	24	24
60-d wt	16.4 ± 0.39^{z}	13.8 ± 0.43^{y}	13.1 ± 0.18^{wy}	14.8 ± 0.76^{wz}	12.6 ± 0.58^{wy}	10.5 ± 0.58^{x}
ADG 60d	0.19 ± 0.01^{z}	0.16 ± 0.01^{y}	0.15 ± 0.003^{wy}	0.18 ± 0.01^{wz}	0.14 ± 0.01^{y}	0.11 ± 0.01^{x}
N for 90d	53	46	246	14	24	24
90d wt	20.4 ± 0.52^{z}	17.8 ± 0.56^{wy}	16.6 ± 0.24^{y}	19.2 ± 1.02^{wz}	16.1 ± 0.78^{xy}	14.6 ± 0.78^{x}
ADG 60d – 90d	0.15 ± 0.001^{z}	0.14 ± 0.01^{z}	0.13 ± 0.01^{z}	0.17 ± 0.03^{z}	0.13 ± 0.020^{z}	0.17 ± 0.02^{z}
ADG 90d	0.18 ± 0.01^{z}	0.16 ± 0.01^{wy}	0.14 ± 0.002^{y}	0.18 ± 0.01^{wz}	0.14 ± 0.01^{xy}	0.12 ± 0.01^{x}

a Information is in the format Birth type – rearing type, SN – single, TW – twin, TR – triplet.
b 60-d and 90-d wts are in kg and ADG is in kg/d.
wxyz Means in the same row with different superscripts differ significantly (*P<0.01*).

90 d traits

Weight at 90 d was significantly *(P < 0.0001)* affected by rearing type, sex, and age. Season also had a significant effect *(P = 0.006)* on 90-d weight. Buck kids were heavier than doe kids at 90 d, as were those born and raised single, while those born and raised triplet were lightest. Kids born in the fall were heavier at 90 d than those born in the spring (18.1 kg ± 0.38 kg vs. 16.9 kg ± 0.34 kg, respectively). Season of birth was significant *(P < 0.0008)* for daily gain between 60 d and 90 d; fall-born kids had higher rates of gain than spring-born kids (Table 2).

Other research supports these findings in relation to average daily gain and 90 d weight. Al-Shorepy et al. (2002) found that males were heavier than females at all ages and single-born-and-raised kids were heavier than twin-born-and-raised kids. They also found that average daily gain to weaning was greater for male kids. Ndlovu and Semela (1996) reported that sex did not impact weight at 90 d, but that kids born in the hot dry season were heavier at 90 d. Wilson and Light (1986) reported that season, type of birth and sex impacted preweaning performance.

Body condition score at weaning was not influenced by type of birth and rearing or sex of kid. However, season of birth had a significant effect *(P < 0.0001)* on body condition score at weaning. Spring-born kids had greater body condition scores than fall-born kids (Table 2) despite being lighter at weaning. The reason for this contrasting relationship is not clear. It would be expected that heavier kids would have higher body condition scores.

FAMACHA© score taken at weaning was not affected by season of birth and sex *(P > 0.20)*. However year of birth *(P = 0.0147)* and type of birth and rearing *(P < 0.0001)* influenced FAMACHA© score taken at weaning. Goats that were born and raised single had better FAMACHA© scores (2.1 ± 0.12) than those of other groups. Kids born and raised as triplets had the worst FAMACHA© score (3.0 ± 0.13) of all birth/rearing types. More research will be needed to determine the reason for this difference.

Survival to weaning

Survival is a very economically important trait and has been shown to be impacted by environmental factors. In this study, survival to weaning was not impacted by season of birth. Number of kids born alive, alive at weaning and percent of loss that occurred between 60 d and 90 d or from birth to 60 d are presented in Table 5. Birth type was the only trait that significantly *(P <0.0001)* influenced survival with triplet-born kids less likely to survive to weaning than single- or twin-born kids. Average temperatures and precipitation for each

Table 5. Survival through weaning.

Season	Number born Alive	Alive at Weaning (%)	% loss before 60 d[a]	% loss between 60 d and Weaning[a]	% loss birth to Weaning[a]
Spring	266	212 (79.7)	18.8	1.5	20.3
Fall	252	198 (78.6)	20.6	0.8	21.4

a % loss is the number of kids lost divided by the total born alive.

Table 6. Average weather during kidding season for Frankfort, Kentucky.[a]

	March	April	May	Spring Average	October	November	December	Fall Average
High Temperature	13.32	19.43	23.87	18.87	21.09	13.88	7.22	14.06
Low Temperature	-0.56	5.00	9.99	4.81	6.11	1.11	-3.33	1.30
Precipitation	10.25	9.31	11.70	10.42	6.75	8.45	9.42	8.21

a Long term average high and low temperatures are reported in degrees C and precipitation is in centimeters.

month during the kidding seasons are reported in Table 6. Average temperature was slightly lower in the fall-kidding season, though it was slightly dryer during the study period. There may not have been enough difference in temperature or precipitation to create differences in survival at this location.

Previous research has shown an impact of season of birth on survival. Hailu et al. (2006) and Husain et al. (1995) both reported that kids born in the wet season had higher survival rates. They also reported that kids born and raised single had higher survival rates that those of other birth types. Husain et al. (1995) saw increased survival by male kids while Hailu et al. (2006) reported that male kids had lower survival than female kids. Browning and Leite-Browning (2011) indicated similar impacts due to litter size and sex of kid on survival to the current study.

Conclusions

Meat-goat producers in the upper region of the Southeastern United States should be able to produce kids using different seasons of birth. Kid rate of gain and weight at 90 d were higher in this study for fall-born kids, which also enter the market in the late winter and early spring when typical auction prices are higher. There were no significant differences in death loss due to season of birth in this study, indicating that producers would not suffer higher losses for fall-born kids in this region. Type of birth/rearing and sex of kid did impact weights and rate of gain for kids. Triplet-born kids were less likely to survive to weaning than single- or twin-born kids when kept on the birth dam. This may indicate a need to look at fostering one of the triplet-born kids to improve overall survival rate to weaning. More research is needed to determine the economic differences between kidding seasons as feeding of stored forages and grains can potentially result in higher cost of production for fall and winter kidding, but kids are ready for market at higher prices if born in fall and early winter.

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Efficacy of Pregnancy-Specific Protein B Assay to Detect Pregnancy and Lambing Rates in Sheep

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Summary

Early and accurate identification of pregnancy and lambing rate provides sheep producers many advantages for management decisions that improve flock productivity. The objective of this study was to investigate the efficacy of a commercial pregnancy-specific protein B (PSPB) ELISA assay to predict pregnancy and lambing rate in sheep. On days 20, 25, 30, 40, and 60 postbreeding, blood samples were collected from Columbia and Hampshire ewes. Dorset and Katahdin ewes were sampled 49, 63, and 77 days post ram introduction. Lambing records were used to verify date of conception. Samples were processed using the quantitative BioPRYN®assay for sheep and goat. BioPRYN® classification was 99 percent accurate for pregnant ewes tested after the first month of gestation (i.e., greater than 30 days). For ewes that did not lamb, 90 percent were classified as open and the remaining 10 percent were either misdiagnosed or lost the pregnancy prior to parturition. Ewes carrying multiple pregnancies had greater serum PSPB concentrations than singleton pregnancies from d 40 to d 69 of pregnancy. Effect of breed was detected for serum concentrations of PSPB from d 40 to d 79 of pregnancy. This research indicates that the BioPRYN® test is an effective tool to identify pregnancy in sheep. This test could provide estimates of lambing rates; however, variation in PSPB concentrations due to stage of pregnancy and breed of sheep must be factored into this analysis.

Key words: Pregnancy-Specific Protein B, Sheep, Fetal Age, Pregnancy Rate, Breed

Introduction

Accurate identification of pregnancy status and lambing rate in sheep provides managers several options to increase flock productivity, including reducing management requirements by culling non-pregnant ewes and feeding ewes appropriate diets based on fetal age and number of offspring. By identifying the pregnancy status of ewe lambs (i.e., 9 months of age), producers have the opportunity to market the ewes at a younger age rather than waiting until they are older and classified as mutton (i.e., 12 months of age). Daily feed requirements are 50 percent and 80 percent greater during late gestation compared to maintenance for single and twin-bearing ewes, respectively (NRC, 2007). Additionally, neonatal lamb loss was 85 percent greater in ewes that gave birth to multiple lambs rather than singles (Rowland et al., 1992); therefore, to improve lamb survival, twin-bearing ewes can be provided separate shed-lambing facilities or a separate lambing paddock depending upon the management system.

Ultrasonic imaging is the most common method to determine pregnancy status in the commercial sheep industry, but is often expensive due to the equipment and trained technicians required. In contrast, a blood test allows for flock managers to take samples without expensive equipment or trained technicians. BioTracking, LLC developed a commercially available, pregnancy-specific protein B (PSPB) test for pregnancy in ruminants called BioPRYN® (BioTracking, LLC, Moscow, Idaho), which is completed using an enzyme linked immunosorbent assay (ELISA). The objectives of this research were to determine the efficacy of the BioPRYN® test to predict pregnancy depending on stage of pregnancy and to determine if the test can predict lambing rate in sheep.

Materials and Methods

All experimental protocols were approved by the North Dakota State University (NDSU) Animal Care and Use Committee. Columbia and Hampshire ewes (n = 34 and n = 31, respectively) from the NDSU sheep unit were exposed to intact rams equipped with marking harnesses beginning on August 15, 2011. Two weeks prior to breeding, the Columbia and Hampshire ewes were moved to a drylot and received alfalfa hay (3 kg/ewe) and a 14 percent CP concentrate ration (1 kg/ewe) daily. Breeding marks were identified and recorded. On days 20, 25, 30, 40, and 60 post-breeding, blood samples were collected to determine PSPB concentrations. Ewes that rebred were reassigned new bleeding dates based on the most recent breeding mark. Dorset and Katahdin ewes (n = 68 and n = 24, respectively) were managed on smooth-bromegrass and alfalfa-mixed paddocks and were exposed to intact rams on September 27, 2011. All ewes were single-sire mated to rams of similar breed type to the ewe and at a ewe-to-ram ratio not exceeding 35:1. Blood samples were taken from all ewes d 49, d 63, and d 77 post-ram introduction. Lambing records were taken to determine breeding dates and lambing rates.

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

Samples were processed using the quantitative BioPRYN® assay for sheep and goats commercially available through BioLaboratories LLC (Moscow, Idaho). All samples were classified as **open** (less than 15 ng/mL PSPB), **retest** (15 to 30 ng/mL PSPB), or **pregnant** (greater than 30 ng/mL PSPB) based on the standard recommendation from the BioPRYN® test. Concentrations of PSPB for each ewe were determined by least-squares linear regression using known concentrations of purified PSPB as standards.

All samples were classified as either taken from ewes that **did not lamb** (within 150 days post-sample collection) or taken from **pregnant** ewes based on lambing date. Additionally, all samples taken from pregnant ewes were assigned a day of pregnancy number. Day of pregnancy was known for Columbia and Hampshire ewes that lambed within 145 d and 152 d post breeding. However, breeding dates were not collected on Dorset and Katahdin ewes, plus some Hampshire and Columbia ewes lambed to a subsequent breeding event, therefore their breeding date was assigned as 150 d (average gestation of known breeding dates) prior to lambing.

General linear model procedures of SAS (SAS Inst., Inc., Cary, N.C.) were used to analyze serum concentrations of PSPB. To determine the earliest that the BioPRYN® test would detect pregnancy, samples were classified into five subcategories based on BioLaboratories recommendations and prior dates that would be relevant to sheep producers: **1) Did Not Lamb**; samples taken from ewes that did not lamb, **2) < 20 d**; samples taken from 0 days to 19 days post breeding, **3) 20 d - 24 d**; samples taken between from d 20 to d 24 post breeding, **4) 25 d - 29 d**; samples taken from d 25 to d 29 post breeding, and **5) > 30 days**; samples taken from day 30 to day 79 post breeding. These categories were chosen specifically because of prior knowledge of the testing limitation (~30 days), to divide up samples that were taken specifically at 20 d , 25 d, and 30 d, and to include other random samples, for which day of pregnancy was determined based on lambing date. Categorization of testing dates allows producers to make decisions based on days instead of estimations based on a regression equation.

To test the differences in PSPB concentrations among breeds and number of lambs born, samples were divided into ten-day categories through d 80; no samples were taken after this day. This categorization was done to test for differences over time and allow for easy time-point comparisons that are relevant to sheep producer's different management systems. Each block of time was examined independently and actual day of pregnancy was included as a covariate. Least squares means are reported, and differences were considered significant at $P \leq 0.05$.

Results and Discussion

Accuracy by Stage of Pregnancy

The PSPB assay accurately detected 90 percent of ewes that did not lamb (Table 1). We hypothesize that the 10 percent that were classified as recheck or pregnant were pregnant at the time of blood sampling; however, lost the pregnancy prior to parturition. In agreement with our hypothesis, estimates of embryonic or fetal loss have averaged 30 percent in sheep (Bolet, 1986) and 20 percent of embryos have been documented

Table 1. Percentages of samples classified as open, recheck or pregnant by pregnancy-specific protein (PSPB) assay.

	BioPRYN® Classification[1]		
Ewe Pregnancy Status[2]	Open	Recheck	Pregnant
Did Not Lamb	90.00	3.75	6.25
Pregnant			
< 20 d	100.0	0.0	0.0
20 - 24 d	58.3	41.7	0.0
25 - 29 d	0.0	16.7	83.3
> 30 d	0.0	1.4	98.6

[1] BioPRYN® classifications are open (less than 15 ng/mL PSPB), retest (15 to 30 ng/mL PSPB), or pregnant (greater than 30 ng/mL PSPB).
[2] All PSPB samples were included in this table. There were 80, 24, 48, 48, and 365 samples from ewes for each respective row/category (top to bottom).

to be lost after d 25 of pregnancy (Dixon et al., 2007). Although, it was not a part of the original design of the experiment, there were 24 samples taken prior to d 20 of pregnancy, and 100 percent of these samples were classified as open by the BioPRYN® assay. Pregnant ewes were classified as **open** (58 percent) or **recheck** (42 percent) by the assay during the d 20 to d 24 range. From d 25 to d 29 of pregnancy, the assays classified 83 percent and 17 percent of pregnant ewes as pregnant and recheck, respectively. The PSPB assay classified 99 percent of all pregnant ewes as pregnant that were sampled at 30 days or later of pregnancy and the other 1 percent was classified as recheck. This research confirms previous results (Willard et al., 1995) using radioimmunoassay for PSPB and laboratory recommendations that ewes should not be tested prior to 30 days post breeding to confirm pregnancy. Additionally, these results agree with similar PSPB assays conducted in cattle (Romano and Larson, 2010).

Number of Lambs Born

In Table 2, concentrations of PSPB are reported over time, and comparisons are established between ewes that gave birth to single, twin, and triplet births. No differences *(P > 0.09)* were detected for serum PSPB concentration taken from d 0 to d 19 post-estrus among ewes that gave birth to one, two, or three lambs. On d 20 to d 29, serum PSPB concentrations were greater *(P < 0.01)* in ewes that gave birth to twins compared to singletons; whereas, no differences were detected for ewes that gave birth to triplets compared to either single- or twin-bearing ewes (*P = 0.10* and *P = 0.99*, respectively). On d 30 to d 39 and d 70 to d 79, there was a tendency (*P = 0.06* and *P = 0.07*, respectively) for serum PSPB concentrations to be greater for ewes that gave birth to multiple lambs compared to ewes that gave birth to one lamb. From d 40 to d 69, ewes that gave birth to two lambs had on average 25 percent greater *(P < 0.05)* serum PSPB concentrations than ewes that gave birth to one lamb. Regardless of day of pregnancy, no differences *(P > 0.10)* in serum PSPB concentrations were detected between ewes that gave birth to two or three lambs. On day 40 to day 59 and day 70 to day 79, serum PSPB concentrations were greater *(P < 0.05)* for ewes that gave birth to three lambs than ewes that gave birth to one lamb; however, no differences *(P = 0.45)* were detected between d 60 and d 69. In agreement with Willard et al. (1995), these data indicate that single and twin pregnancies can be estimated by PSPB concentrations after d 40 of pregnancy; however, actual age of pregnancy must be known to adjust for changes in PSPB throughout gestation. Our data do not indicate that twin and triplet pregnancies could be predicted. Only 14 ewes gave birth to three lambs (2 Columbia ewes, 5 Dorset ewes, 1 Hampshire ewe, and 6 Katahdin ewes). Too few ewes gave birth to triplets to detect modest differences among multiple pregnancies. However, incomplete pregnancy loss, during or after these samples were collected, is common in sheep (Dixon, 2007), and this could have contributed to added variation in this data set. Wallace et al. (1997) reported that PSPB concentrations increase during rapid placental growth, which implies that PSPB is released by the binucleate cells of the trophectoderm in concentrations proportional to placental mass. Total placental mass is greater in twin versus singleton pregnancies (Alexander, 1974) indicating a rationale for higher PSPB concentrations in twin pregnancies.

On d 0 to d 9, d 20 to d 29, and d 30 to d 39, no differences among breeds *(P > 0.11)* were detected for serum PSPB concentrations (Table 1). On d 10 to d 19, Hampshire ewes had greater *(P < 0.04)* PSPB concentrations than Columbia and Dorset ewes. We are cautious to make any

Table 2. Serum pregnancy-specific protein B (PSPB) concentrations (ng/mL) at different stages of pregnancy in ewes with different numbers of lambs born.

	Number of Lambs Born				
Day of Pregnancy[1]	Single	Twin	Triplet	SEM	*P-value*
0-9	6.2	5.8	-	0.5	0.47
10-19	6.1	3.9	-	1.2	0.05
20-29	23.9[a]	30.7[b]	30.6[ab]	4.3	<0.01
30-39	59.8	68.9	70.9	6.5	0.06
40-49	61.3[a]	77.9[b]	80.8[b]	6.1	<0.01
50-59	55.4[a]	75.8[b]	89.9[b]	9.5	<0.01
60-69	83.6[a]	100.4[b]	100.4[b]	8.4	<0.01
70-79	66.0	85.7	88.4	12.5	0.07

[a,b] Row means with different superscripts differ (*P < 0.05*).
[1] All pregnant ewe PSPB samples were included in this table. There were 11, 12, 97, 77, 105, 51, 98, and 33 samples for each respective row category (top to bottom). If fewer than two samples were taken within a number of lambs born category for a particular day of pregnancy range, means were not presented.

inferences based on this detected difference because the mean values are approximately 1/3 the threshold for pregnancy detection established by BioLaboratories, and only 12 samples were taken during this time period. On d 40 to d 49 and d 60 to d 69, Columbia ewes had greater *(P < 0.03)* concentrations of PSPB than all other breeds. During the same time frame, Dorset ewes had greater *(P < 0.01)* concentration of serum PSPB than Katahdin ewes and Hampshire ewes were not different *(P > 0.09)* from either Dorset or Katahdin. On d 50 to d 59 *(P < 0.02)* and d 70 to d 79 *(P = 0.03)*, Columbia and Dorset ewes had greater concentrations of PSPB in serum than Katahdin ewes. Willard et al. (1995) also reported differences among breeds for PSPB concentrations. Their research indicated that Columbia ewes had greater concentrations of PSPB than Rambouillet and Targhee ewes. It is unknown why differences in PSPB exist among breeds; however, it is likely a result of differences in placental development or production of PSPB per-unit placental mass, as indicated by Wallace et al. (1997). Of the breeds used in this project, Katahdin and Dorset have a much smaller mature size than Columbia and Hampshire ewes. These large differences in BW and variation in blood-volume-per-unit BW could have an impact on PSPB concentrations; however, there does not appear to be a correlation between PSPB concentrations and breed mature size.

Conclusions

This research demonstrates that the BioPRYN® test is an effective tool to detect pregnancy in sheep after the first month of gestation. Tested after 30 days of pregnancy, 99 percent of pregnant ewes were classified as pregnant and only 6.25 percent of ewes that did not lamb were classified as pregnant, which is likely due to fetal loss. Concentrations of PSPB were dependent on stage of pregnancy, breed of sheep, and lambing rate. Differences in PSPB concentrations were detected between singleton and twin-bearing ewes. This test could be used to estimate lambing rate; however, variation in PSPB contributed by breed and age of pregnancy must be quantified. Additionally, if PSPB can serve as a non-invasive measure of placental development, it could improve scientific investigation towards enhancing or alleviating improper placental/fetal development.

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Table 3. Serum pregnancy-specific protein B (PSPB) concentrations in ewes from different breeds at different stages of pregnancy.

	Ewe Breed					
Day of Pregnancy[1]	Columbia	Dorset	Hampshire	Katahdin	SEM	*P-value*
0-9	6.4	-	5.5	-	0.6	0.17
10-19	5.1[b]	-	7.0[a]	-	1.0	<0.01
20-29	30.8	22.3	28.3	32.2	9.7	0.50
30-39	71.9	71.5	62.3	60.5	7.1	0.11
40-49	89.3[a]	75.5[b]	69.1[bc]	59.5[c]	6.9	<0.01
50-59	110.8[a]	95.6[a]	-	63.5[b]	18.8	<0.01
60-69	121.1[a]	98.1[b]	86.5[bc]	73.4[c]	9.2	<0.01
70-79	-	90.8[a]	-	69.3[b]	9.1	0.03

[a,b,c] Row means with different superscripts differ (*P < 0.05*) within a row.

[1] All pregnant ewe PSPB samples were included in this table. There were 11, 12, 97, 77, 105, 51, 98, and 33 samples for each respective row category (top to bottom). If there were fewer than two samples within a breed for a particular day of pregnancy range, means were not presented.

