IMPACTS OF SUPPLEMENTAL ARGININE ON EWE REPRODUCTIVE PERFORMANCE

A Thesis
Submitted to the Graduate Faculty
of the
North Dakota State University
of Agriculture and Applied Science

By

Alison Ryan Crane

In Partial Fulfillment
for the Degree of
MASTER OF SCIENCE

Major Department:
Animal Sciences

April 2014

Fargo, North Dakota

North Dakota State University
Graduate School

Title

IMPACTS OF SUPPLEMENTAL ARGININE ON EWE REPRODUCTIVE PERFORMANCE

By

Alison Ryan Crane

The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

SUPERVISORY COMMITTEE:

Dr. Reid Redden
Co-Chair

Dr. Christopher S. Schauer
Co-Chair

Dr. Joel Caton

Dr. Kevin Sedivec

Approved:

04/24/2014
Date

Dr. Greg Lardy
Department Chair

ABSTRACT

The objective of this study was to determine the effects of injectable and oral arginine (Arg) supplementation provided 14 d post-breeding on reproductive performance of fall lambing ewes. Upon estrus detection (d 0) ewes were randomly assigned to one of six treatments for a 14-d treatment period: injectable saline **(CON**; n = 25), injectable alanine (**IVALA**; n = 20), injectable arginine (**IVARG**; n = 23), oral rumen-protected Arg (**RPARG**; n = 20), oral soybean meal (**SBM**; n = 23), or oral fishmeal (**FM**; n = 24). Weaning rates were higher ($P < 0.05$) in Arg supplemented ewes. Plasma progesterone and serum Arg concentrations exhibited a treatment and day effect ($P < 0.05$), but no treatment × day interaction was observed ($P > 0.05$). In contrast to previous research, supplemental Arg during the first 14 d of pregnancy did not improve pregnancy or lambing rates, however, IVARG positively impacted weaning rates.

Key Words: arginine, progesterone, reproduction, sheep

ACKNOWLEDGEMENTS

I would like to thank the Department of Animal Sciences and the department head, Dr. Greg Lardy, for the opportunity to further my education. I would also like to thank my committee for being very supportive through this process. Thank you to Drs. Jay Daniel, Ricardo Zanella and Kyle Caires for providing me with the interest and guidance to pursue graduate school and for the confidence to believe I could do it.

I would like to thank Dr. Marc Bauer for answering a multitude of questions about animal nutrition, beets and debates. Our conversations taught me about asking the right questions and how I can better learn from asking them. Dr. Joel Caton, you have been like a home away from home father for me. Thank you for the tea, the amazing coffees and for the stimulating conversation. Dr. Larry Reynolds, thank you for appreciating my diverse background. You always make me feel appreciated in a field where I am always wondering if I belong.

I cannot thank my two advisers, Drs. Reid Redden and Christopher Schauer, enough for not only believing in me and allowing me to work for them, but to also push me above and beyond my expectations for myself. You both have pushed me to live up to my full potential that I did not realize I had. Thank you for instilling a willingness to learn, ask questions, and showing me how much I have to learn. The guidance I have received from both of you led to an amazing journey. The past two years have been wonderful and I would not have made it through without the jokes, trips, and endless picking. After these two years, I treasure and hope to never lose these friendships both professionally and personally.

I would also like to thank the staff of the Hettinger Research Extension Center. To the 'girls' for helping me with my constant technology difficulties and our lunch dates. To Don and Donnie for giving me a hard time and letting me hang on the farm crew when I could not stand

another day of writing. To Dave for being Dave and keeping me sane, helping me lamb out my ewes and always being there whenever I needed him and teaching me about the North Dakota sheep industry. To my lovely ladies on the farm crew and my technicians, Rachael Lagein, Kelsie Egeland and Becca Turnquist, for keeping me going through my projects, I could not have done it without you girls. I had a blast.

Thank you to Marsha Kapphahn for running endless amounts of samples and to Jim Kirsch and Sheri Dorsam for walking me through how to use the lab equipment and always being there to answer my endless amount of questions and therapy sessions. Thank you Roberta Dahlen and Dr. Lauren Hanna for guiding me through SAS. You both are amazing.

Thank you to my fellow graduate students for helping me make it through every day, I honestly could not have made it these past two years without all of you. Thank you to Casie for pushing me to read everything I could get my hands on and for showing me that lab work can be fun and pretty cool. To Ben Klinkner for always keeping me laughing in my first year in Fargo and showing me the ropes. Beth Mordhorst, you're awesome. I have a lot of great Fargo memories thanks to you. To Jeff Stackhouse for being the coolest guy I've ever met and taking me on my first duck and pheasant hunt and for showing me all of the good fishing spots around Hettinger.

I would also like to thank members of the sheep industry nationwide, but especially those in the midwest and western regions. I have received guidance from some of the greatest in the business and your patience and sharing of knowledge is greatly appreciated. Dr. Lisa Surber, you have given me guidance as a colleague, a mentor and a friend and I couldn't have made it through this process without you. Thank you for always being there for me. Mike Hagens, you have been my best friend here in North Dakota, from submerging me into the shearing family

and lifestyle, to letting me follow around your awesome shearing crew and teaching me about wool, to rescuing me when I didn't have a car to drive. You have shown me what true friends do for one another and I could honestly never repay you, thank you. Alex Moser, thank you for listening to my sometimes (probably mostly) endless rants about different parts of my thesis and letting me bounce ideas off of you. I could not have done this without you.

And last, I dedicate my thesis to my family. My sister and best friend, Mary Catherine, you have always been there for me and convinced me I'm stronger than I am. Thank you for being an amazing big sister. And to my other 'sister', Bailee, for the phone calls getting my mind off of life and the therapeutic trips to the lake when I come home. To my parents, Larry and Nancy Crane, you are the two most amazing people I've ever met. Dad, you've always gone above and beyond to make sure I am doing what I love and that I am happy, but you forget, I learned from the best. You also taught me honesty and what it means to work hard and do your best. I never would have made it this far without you. You showed me that the most important thing in life is family and being happy with what you have done. Mom, thank you for talking to me at all hours of the day, being there for me in my lowest of lows, and always believing in me and pushing me to be better than I ever thought I could be. I'm lucky enough for my parents to have become my best friends and the two of you mean the world to me. I hope I have made you both proud. And to my grandmother, Florence Standridge, the strongest person I know and ever will. I strive every day to be more like you, thank you for somehow convincing me that an education is a privilege I deserve, rather than a burden to complete. You have instilled in me what a true Southern lady is and I thank you for that. Thank you for your 'Southern Comfort" care packages that kept me from getting homesick. I thank God every day for my wonderful and

beautiful family. You all mean the world to me and I thank you for your unconditional and never-ending support.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

µL.....microliter

ADF..... acid detergent fiber

Arg ..... arginine

BW .....body weight

Ca .....calcium

CIDR .....controlled internal drug release

Cit..... citrulline

CON ..... control

CP..... crude protein

Cu.....copper

d.....day

DM .....dry matter

FM..... fish meal

G..... gauge

g..... gram

GABA .....γ-amino-N-butyric acid

GLM.....general linear model

h..... hour

Hyl2..... hydroxylysine-2

IVALA ..... intravenous alanine

IVARG.....intravenous arginine

kg.....kilogram

LSD ..... least significant difference

MSE ..... mean square error

mg ..... milligram

min ..... minute

mL ..... milliliter

NDF ..... neutral detergent fiber

NRC ..... National Research Council

Orn ..... ornithine

ODC ..... ornithine decarboxylase

P ..... phosphorus

ppm ..... parts per million

RPARG ..... rumen-protected arginine

SAS ..... Statistical Analytical Software

SBM ..... soybean meal

SEM ..... standard error of the mean

TDN ..... total digestible nutrients

LIST OF APPENDIX TABLES

CHAPTER 1. INTRODUCTION AND LITERATURE REVIEW

Introduction

Breeding sheep inventory in the United States on January 1, 2014 totaled 3.88 million head, down 2 % from 2013 [National Agriculture Statistics Service (NASS), 2014]. Current reproduction research in sheep has focused primarily on increasing lamb production by increasing the number of lambs born per ewe, therefore increasing potential returns for the producer. However, the 2013 U.S. lamb crop totaled 3.37 million head, down 2% from 2012, with a weaning rate of 107 lambs per 100 ewes one year or older, also down 2 % from the previous year (NASS, 2014). With this in mind, new research should be focused on a feasible resolution to increase reproductive efficiency in sheep.

Past studies conducted in early-life nutrition in sheep began in the 1970s and were focused on the early neonatal period and the latter part of pregnancy. These time periods were chosen because the nutrient demands on the fetus are highest at these stages of reproduction (Robinson et al., 1983). Sensitivity to nutritional status was observed by Parr et al. (1992), who reported that ewes fed two times their maintenance requirements after breeding had a pregnancy rate of 48%, while those ewes fed rations calculated to maintain live weight or fed half the maintenance ration during this same time had pregnancy rates ranging from 60 to 68%. These findings showed correct nutritional status is essential in early pregnancy, especially in the time before the conceptus is present in the uterus or d 13 of pregnancy, if lifespan of the CL is to be extended beyond d 14 (Moor et al., 1960). Thus, d 14 is when maternal recognition occurs (Moor et al., 1960). Previous research has indicated if maternal blood flow can be increased to the conceptus prior to maternal recognition of pregnancy (d 14), embryonic loss might be

decreased (Luther et al., 2009; Saevre et al., 2011a). However, information concerning the underlying mechanisms that effect embryonic loss in large animal species is largely lacking.

Embryonic and fetal deaths during pregnancy account for 25 to 50% of the total number of fertilized ova in the ewe (Knights et al., 2003; Dixon et al., 2007). The majority of embryonic loss has been reported to occur before d 18 (Hulet et al., 1956; Moore et al., 1960; Quinlivan, 1966). However, Dixon et al. (2007) reported that 19.9% of ewes experience late embryonic loss, fetal loss, or both; and 21.2% of the embryos or fetuses were lost from d 25 to term. In multiple birth type pregnancies, loss of individual embryos or fetuses can occur without a total loss of pregnancy (Rhind et al., 1980; Schrick and Inskeep, 1993). Additionally, a small percentage of embryos are inherently non-viable in the ewe (Wilmut et al., 1986), which would suggest that a proportion of early embryonic losses could be prevented. A better understanding of preventing embryonic loss would provide a method to increase livestock productivity.

Recently, low circulating concentrations of progesterone, estrogen, and vascular endothelial growth factor in the ewe have been identified as factors affecting prenatal losses (Dixon et al., 2007). These factors are important for optimizing the uterine environment and ensuring proper development of the placenta during early pregnancy (Nephew et al., 1991; Spencer et al., 2004).

The amino acid L-arginine (Arg) is important for the synthesis of polyamines and nitric oxide, both of which are essential for proper development of the embryo and placenta. Gestating sows supplemented with Arg achieved a 22% increase in live piglets born (11.4 vs. 9.4, $P < 0.03$, respectively; Mateo et al., 2007). Furthermore, pregnant rats supplemented with 1.3% Arg-HCl throughout pregnancy or between d 1 and 7 of gestation had increased embryonic survival and litter size by 30% (Zeng et al., 2008). It is reasonable to hypothesize that supplementation with

Arg would have beneficial impacts on optimizing the uterine environment for ensuring early embryonic survival in sheep.

The most important factor effecting success of most commercial sheep operations is reproductive efficiency (Lupton, 1998); however, it is also one of the most difficult factors to improve. Currently, the sheep industry is focused on the need for improved reproductive efficiency by increasing the number of lambs born per ewe while improving the growth efficiency of those offspring. New advancements in animal science research offer the possibility for significant improvements in efficiency of lamb production.

Reproduction in the Ewe

Sheep are one of many mammals that have synchronized reproductive activity, which is coordinated by changes in length of day (Woodfill et al., 1991; Malpaux et al., 1996; Barrell et al., 2000). These seasonal patterns of reproduction are coordinated to allow parturition to occur in spring months, which commonly produce more and higher quality forage that increases the likelihood for offspring to thrive. Most sheep breeds exhibit a short day breeding pattern when located in mid to high latitudes, shifting parturition's occurrence to the spring of the year, allowing offspring to grow and develop during summer months while availability of forage is increased and weather is optimal before declining into the winter season. However, in sub-tropical and tropical environments, there is a tendency for ewes to breed throughout the year with forage quality being the main controlling factor in breeding activity (Rosa and Bryant, 2003). Seasonality in sheep is controlled by multiple environmental cues, including photoperiod and geographic location (Vivien-Roels and Pévet, 1983).

The main environmental cue controlling seasonality in sheep is photoperiod via neural and humoral processes (Malpaux et al., 1996). As light enters the retina of the eye, presynaptic

neurons are stimulated in the suprachiasmatic nucleus; this stimulation sends signals to the superior cervical ganglion initiating the firing of the postganglionic neurons (Senger, 2005). The message regulates melatonin secretion by the postganglionic neurons synapse along with inhibitory neurons connecting with pinealocytes (Malpaux et al., 1996; Senger, 2005). During short photoperiods, signal transmission is decreased, which decreases the inhibition on the pineal gland and increases the amount of melatonin released. The activity of the hypothalamo-hypophyseal and gonadal axis is regulated by the duration of melatonin secretion (Karsch et al., 1989). Melatonin then stimulates the release of gonadotropin releasing hormone, or GnRH.

Ewes are considered to be polyestrus and usually exhibit an estrous cycle every 17 d during the fall of the year (Schillo, 2009). The estrous cycle is divided into four stages: estrus, metestrus, diestrus and proestrus. These are then grouped into two main phases: the follicular phase and luteal phase. During the follicular phase, the CL or corpora lutea regresses and ovulation occurs, thus including proestrus and estrus. The luteal phase, including metestrus and diestrus, is described as the time from ovulation to the time of regression of the CL.

Maternal Recognition of Pregnancy

In order for normal development and differentiation of the embryo to occur, communication between the embryo and maternal system must be established following conception. Maternal recognition of pregnancy occurs at approximately d 13 following conception. The migration of the embryo has been reported to occur between d 12 and 14 of gestation in the ewe with attachment of the trophoblast occurring at d 16 to 17 (Nephew et al., 1989). The synthesis of estradiol is closely associated with the intrauterine migration of embryos (Nephew et al., 1989). Proper development of the embryo initially depends on the maternal and paternal genes. When the conceptus elongates from blastocyst to the filamentous form, interferon

tau (IFNτ) is produced, which is responsible for the antiluteolytic signal for the recognition of pregnancy (Spencer and Bazer, 2002). The maintenance of the CL, the primary structure responsible for progesterone production during early pregnancy, is allowed only because of the presence of IFNτ, which also inhibits the pulsatile production of PGF2α (Bazer et al., 1998). The major protein, IFNτ, is secreted by the trophectoderm of the peri-implantation conceptus between d 10 and 21 of pregnancy in sheep (Bazer et al., 1998). The concentration of IFNτ increases around d 12 to 13 as the embryo takes on a more filamentous shape. This indicates that IFNτ is important in the recognition of pregnancy along with progesterone and its suppression of PGF2α.

Progesterone is also necessary for the secretion of histotroph by the uterine glands, which helps nourish the conceptus during the peri-implantation period (Spencer et al., 2004). Histrotroph contains enzymes, growth factors, cytokines, lymphokines, hormones and transport proteins (Spencer et al., 2004), all of which have been shown to ensure proper development and survival of the embryo in humans (Burton et al., 2002), primates (Bazer et al., 1979; Roberts and Bazer, 1988; Carson et al., 2000; Gray et al., 2001a) and sheep (Lawson et al., 1983; Flechon et al., 1986; Gray et al., 2001b; Gray et al., 2002).

Reproductive Loss in the Ewe

Reproductive loss accounts for one of the largest economic inefficiencies in any livestock operation, although often unnoticed (Bradford and Meyer, 1986). The majority of prenatal loss occurs before d 18 of gestation (Hulet et al., 1956; Moore et al., 1960; Quinlivan, 1966). Early development could also play a role in altering losses later in gestation. For example, epigenetics caused by events such as adverse uterine environment or poor maternal nutrition status (Bloomfield, 2011). As stated previously, research by Dixon et al. (2007) reported that

approximately 19.9% of all ewes experience late embryonic loss, fetal loss, or both; and 21.2% of the embryos or fetuses were lost from d 25 to term. Loss of individual embryos can occur without a complete loss of pregnancy, such as in the case of multiple fetuses (Rhind et al., 1980; Schrick and Inskeep, 1993).

It has been reported that 30% of fertilized ova in sheep are not represented by live births, resulting in frequent, but unrecognized as a form of flock production loss (Bolet, 1986; Knights et al., 2003; Dixon et al., 2007). Some research has concluded that the majority of embryonic loss in the ewe occurs around the time of maternal recognition of pregnancy (Cross, 2001). Prenatal loss in the ewe has also been linked to abnormal circulating concentrations of progesterone, estrogen and vascular endothelial growth factor (Dixon et al., 2007). Strategies to enhance prenatal growth and survival could clearly have a major economic impact in the sheep industry.

Functions of Arginine

Arginine is an essential amino acid for young, growing mammals and carnivores and conditionally indispensable for young ruminants (Ball et al., 2007). Requirements for Arg are increased in the neonate and necessary to promote postnatal growth and development; however, as development progresses, the need decreases. The small intestine is a major source of circulating citrulline for endogenous synthesis of Arg in the rat (Windmueller and Spaeth, 1981). Arginine was also identified as a precursor for nitrite/nitrate synthesis and nitric oxide (NO), which is the endothelium-derived relaxing factor essential for vascular tone and hemodynamics (Wu and Morris, 1998; Figure 1.1). Below is a listing of the many functions and pathways of Arg synthesis and metabolism:

- Nitric oxide was identified as the active intermediate of the Arg/NO pathway in macrophages and endothelial cells (Wu and Morris, 1998).
- Citrulline is converted to Arg in the kidney via Argininosuccinate synthase (ASS) and Argininosuccinate lyase (ASL; Wu and Knabe, 1995). Enzymes ASS and ASL convert citrulline to Arg in the cytosol.
- Glutamine and citrulline decrease when there is a decrease in phosphate-dependent glutaminase (PDG), pyrroline-5-carboxylate synthase (P-5-C) and carbamoyl phosphate synthase I (CPS I). Enzymes PDG, P-5-C, ornithine carbamoyltransferase (OCT), and CPS I catalyze citrulline formation from glutamine in the mitochondria.
- The conversion of citrulline to Arginine decreases with a decrease of ASS and ASL.
- Glutamine serves as major fuel source for neonatal pig enterocytes and provides all carbon and nitrogen molecules for Arg synthesis.
- The synthesis of Arg from glutamine involves two portions of enterocyte cells, the mitochondria and cytosol. The process involves many enzymes including: PDG, P-5-C, ornithine aminotransferase (OAT), OCT, CPS I, ASS and ASL.

However, the pig is the only species of mammal to release endogenously synthesized Arg into venous circulation. Sixty percent of the net synthesized Arg in adult mammals occurs in the kidney within proximal convoluted tubules; whereby, citrulline is transferred to Arg via ASS and ASL. This is limited by the amount of citrulline produced by the small intestine (Osowska et al., 2004).

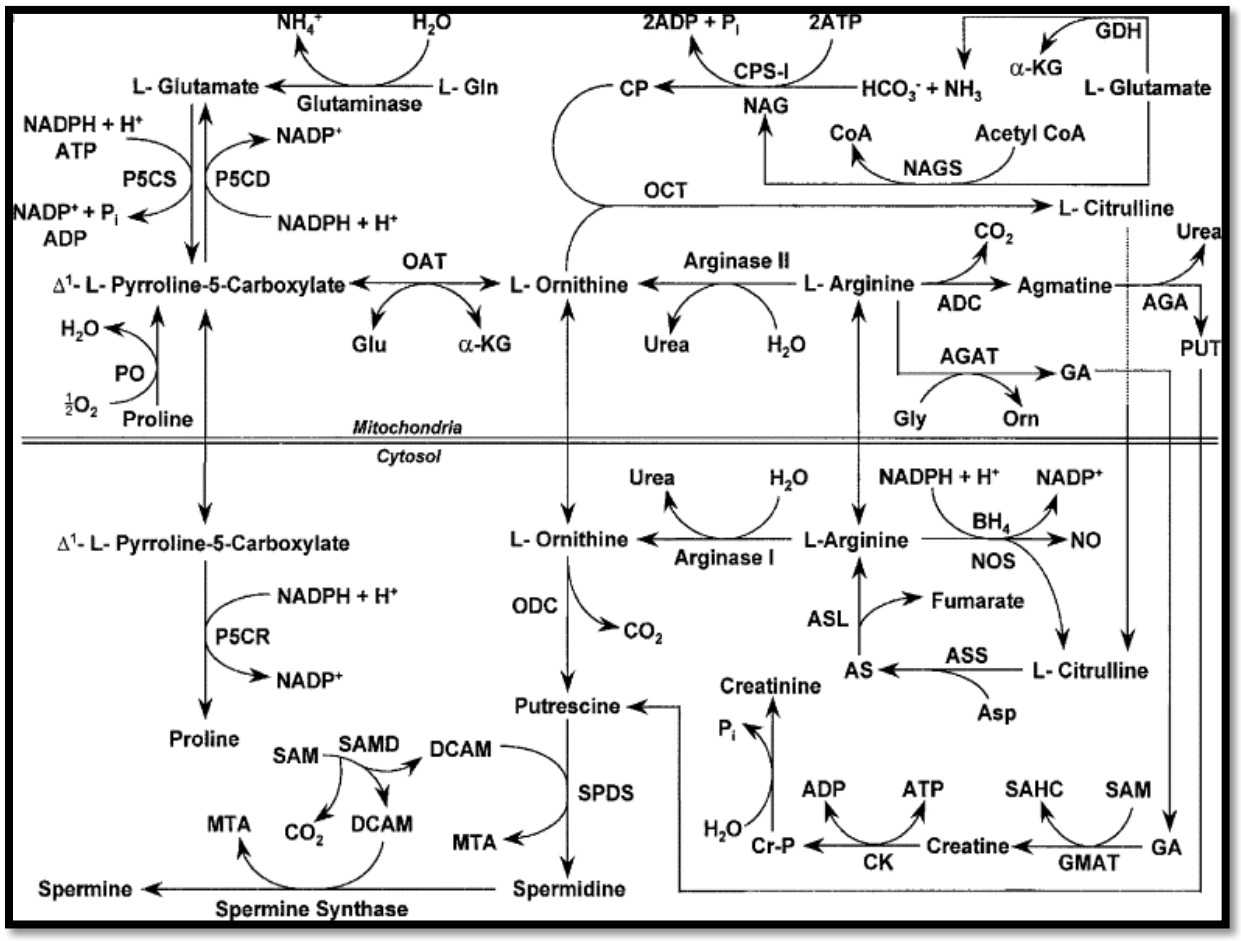

Figure 1.1. Arginine metabolism in mammals. Reprinted from Biomedicine and Pharmacotherapy (Flynn et al., 2002).

Argininase is the enzyme required for regulatory availability of Arg and for the synthesis of NO, polyamines, proline and glutamate. Type I Argininase is a cytosolic enzyme expressed in high concentrations in the liver as part of the urea cycle. Within the urea cycle it detoxifies waste nitrogen in the liver (Wu and Morris, 1998). Type II Argininase is a mitochondrial enzyme,

which is expressed in the kidney, brain, small intestine, mammary gland and macrophages (Wu and Morris, 1998). Argininase competes for Arg for synthesis of NO. At high levels of Argininase the amount of needed Arg is limited for NO synthesis by cells. Argininase is also important for wound healing. Polyamines, which are critical for cell proliferation and differentiation, are synthesized from ornithine via ornithine decarboxylase (ODC) and argininase (Wu and Morris, 1998). When levels of argininase are decreased, no proliferation occurs. In newborns, levels of argininase activity are low in the enterocytes; therefore, the amount of proliferation from polyamine synthesis in the small intestine is decreased (Wu and Morris, 1998). However, its activity increases with age along with polyamine synthesis. This increase is due in part to glucocorticoid introduction.

Proline (Pro) is a major product of Arg catabolism in the enterocyte of post-weaning pigs (this varies with age and species), but is virtually undetectable in newborns (Wu, 1997). Proline is an essential dietary amino acid for the neonatal piglet, but the requirement decreases with level of age and development (Wu, 1997). Proline synthesis in the mammary gland is regulated by argininase activity. This enzyme is required for the synthesis of Pro from Arg along with OAT and P-5-C reductase. Output of Pro in milk exceeds uptake of Pro by lactating mammary gland, although Arg uptake exceeds output. The level of Pro produced is dependent on Arg levels rather than glutamate due to the absence of P-5-C synthase. This process does however provide a substrate for glutamate synthase. Arginine decarboxylase, found in the brain, liver, kidney, adrenal gland, macrophages and the small intestine; however, in the mitochondria it is responsible for the production of carbon dioxide and agmatine (a product of decarboxylation and intermediate of polyamine synthesis) from Arg (Wu, 1997).

Nitric oxide and the enzyme NO synthase both play a role in steroidogenesis, ovulation, embryo implantation and maintenance of pregnancy (Gouge, 1998; Manser, 2004). Nitric oxide relaxes vascular smooth muscle and is produced by endothelial cells (Ignarro et al., 1987). Nitric oxide synthesis is the result of the oxidation of Arg to citrulline by NO synthase (Figure 1.1). Nitric oxide synthase play a regulatory role in Arg degradation to NO. The enzymes are regulated by a Ca^{2+}/calmoduline feedback mechanism (Ignarro et al., 1987). Relaxation of vascular smooth muscle is caused by the activation of the cyclic guanosine monophosphate pathway (Moncada and Higgs, 1993). During pregnancy in rodents, inhibition of NO synthase expressed higher incidence of embryonic mortality (Chwalisz et al., 1999). Arginine supplemented pigs exhibited a 22% increase in the number of live piglets born (Mateo, 2007). Zeng (2008) reported that Arg increased embryonic survival and litter size in rats by 30% whether supplemented throughout or between d 1 and 7 of pregnancy with 1.3% Arg •HCl. Arginine treatment in late pregnancy increases transport of nutrients to the unborn lamb (Thureen, 2002), enhances fetal protein levels in sheep, and increases lamb BW at birth (De Boo, 2005).

Arginine plays an essential role in the synthesis of polyamines (Table 1.1). Polyamines are essential for cell growth and differentiation, including spermatogenesis. In pigs, 1% Arg •HCl was given to sexually active boars for 30 d and although it had no effects on semen volume, Arg and polyamine concentrations were increased (43 and 63%, respectively; Wu et al., 2009). Wu et al. (2009) also reported sperm concentrations increased by 18% and motility by 8%. In rats fed an Arg-free diet, an increase in fetal reabsorptions, intrauterine growth retardation, increase in embryonic loss and a decrease in number of live fetuses born was observed (Greenburg et al.,

1997). Arginine given to rats via drinking water prevented hypoxia-induced fetal growth retardation (Vosatka et al., 1998).

Table 1.1. Roles of Arginine in reproduction. Adapted from Wu et al., 2009

Roles of Arginine on reproduction	Effect	Mediators
Embryo implantation, survival and growth	Increase	Nitric oxide, polyamines and protein synthesis
Fetal survival, growth and health	Increase	Nitric oxide, polyamines and protein synthesis
Ovulation, ovarian steroidogenesis and oocyte quality	Increase	Nitric oxide and polyamines
Placental angiogenesis, growth and function	Increase	Nitric oxide, polyamines and protein synthesis
Spermatogenesis, sperm quality and male fertility	Increase	Nitric oxide, polyamines and protein synthesis
Uterine contractility and preterm labor	Decrease	Nitric Oxide
Erectile dysfunction	Decrease	Nitric Oxide
Preeclampsia in human pregnancy and animal models	Decrease	Nitric Oxide

Supplementation of Arginine

Wu et al. (1998) stated that Arg is a dispensable (non-essential) amino acid for healthy adult humans, but is essential for young, growing mammals and carnivores, along with being conditionally essential in adult humans and other animals, particularly in cases of disease or trauma. Wu et al. (1998) reports that in adult animals the majority of endogenous Arg synthesis involves an inter-organ pathway, in which the small intestine releases citrulline into the blood circulation, which is then extracted primarily by the kidney for conversion to Arg. However, Arg synthesis and fluxes are underestimated and not completely understood in many tissues. For example, the high rates of Arg flux within the urea cycle in the liver are virtually invisible in most studies of plasma Arg flux. The ovine placenta contains a high amount of arginase activity (Kwon et al., 2004) that would catabolize Arg. The placental transfer of citrulline and its storage in allantoic fluid provide an effective strategy to conserve Arg in the ovine conceptus (Wu et al.,

2006). During the first half of pregnancy, when the growth of the placenta is most rapid, there is an unusual abundance of the Arg-family of AA in fetal fluids of the ovine and porcine (Kwon et al., 2003a, 2004; Self et al., 2004; Wu et al., 2005). These findings illustrate the crucial roles of Arg-dependent pathways in conceptus growth and development in two diverse species models (Wu et al., 2006). Wu et al. (2004) noted that studies in pregnant pigs and sheep indicated impaired synthesis of NO and polyamines in the conceptuses of underfed and overfed dams. Dietary protein deficiency reduced the availability of Arg and ornithine in maternal plasma, fetal plasma, amniotic fluid, and allantoic fluid as well as placental synthesis of NO and polyamines in pregnant pigs (Wu and Morris, 1998; Wu et al., 1998), with similar results in underfed ewes (Kwon et al., 2004).

Previously, Arg has been provided through injection or supplemented in the feed. However, with ruminant species, protein is predominately degraded in the rumen; therefore, not allowing specific nutrients such as Arg to reach the small intestine at similar concentrations to the feed for absorption.

Injectable Arginine

Thureen et al. (2002) showed that Arg injections during late pregnancy in ewes increased transport of nutrients to the fetus, while De Boo et al. (2005) reported enhanced lamb birth weight with injections of Arg during late gestation. In a trial conducted by Luther et al. (2009), ewes receiving injectable Arg from the time of standing estrus to d 12 of pregnancy lost fewer embryos during early pregnancy and ultimately gave birth to more lambs per ewe. In this trial, Arg appeared to enhance early uterine environment, making it more ideal for embryonic survival with increased levels of progesterone. Lassala et al. (2011) reported ewes with multiple fetuses provided with injectable Arg between d 100 and 121 of gestation had reduced percentage of lambs born dead (23%) and an increased percentage of lambs born alive (59%). Lassala et al.

(2011) also found that birth weights of quadruplet lambs were enhanced by 23% when ewes were given injectable Arg without affecting maternal BW; furthermore, improved pregnancy outcome was associated with the increase in maternal plasma concentrations of Arg, ornithine, cysteine, and proline, as well as a decrease in circulating levels of ammonia and β-hydroxybutyrate. Lassala et al. (2010) evaluated an ovine intrauterine growth restriction (IUGR) model to test the hypothesis that parental administration of L-Arg is effective in enhancing fetal growth. Beginning on d 28 of gestation, ewes were fed a diet providing 100% (CON) or 50 % (underfed) of NRC-recommended nutrient requirements. Between d 60 of gestation and parturition underfed ewes received IV injections of saline or 155 μmol Arg-HCl/kg BW 3 times daily; whereas, control-fed ewes received only saline. The birth weights of lambs from saline-infused underfed ewes were 23% lower than those of lambs from control-fed dams. Administration of Arg to underfed ewes increased concentrations of Arg (69%), ornithine (55%), proline (29%), and methionine (37%) in maternal serum and enhanced birth weights of lambs by 21% compared with saline-infused underfed ewes. These results indicate that parenteral administration of Arg to underfed ewes prevented fetal growth restriction and provide support for its clinical use to alleviate IUGR in humans.

Saevre et al. (2011a) also studied the effects injectable Arg in pregnant ewes when supplemented from d 9 to 14 post-estrus. In this study, the authors report that ewes injected with Arg had greater pregnancy rates, increased circulating serum Arg and ornithine concentrations, along with elevated vascular resistance in peripheral blood flow. However, differing from other trials, Saevre et al. (2011a) found that ewes given injectable Arg had lower concentrations of progesterone relative to the control ewes on d 9 and 10 of gestation, but were similar for the remaining treatment period. Since Arg treated ewes had greater pregnancy rates in this trial

compared to the control ewes, progesterone levels were not believed to be low enough to lead to embryonic loss as observed by Dixon et al. (2007).

Supplemental Arginine in the Diet to Monogastrics

Supplemental Arg, when given to sows in the diet from d 30 of gestation until d 110, has been reported to increase the number of live piglets born per sow (Mateo et al., 2007); most likely due to an increase in nutrient delivery to the developing embryo. Piglets born to Arg supplemented sows in the trial by Mateo et al. (2007) also had increased live litter birth weights when compared to the control group sows. At d 90 and 110 of gestation, sows supplemented with Arg had lower plasma urea nitrogen concentrations than control gilts (Mateo et al., 2007). A tendency for decreased plasma urea nitrogen concentrations in Arg supplemented animals have been observed in other trials (De Boo et al., 2007; Mateo et al., 2008; Zeng et al., 2008). Mateo et al. (2007) reports that Arg supplementation may reduce whole-body amino acid degradation and, thus, urea production as well as the endogenous synthesis of glutamine from branched-chain amino acids and ammonia. In rats supplemented Arg between d 1 and 7 of gestation, Zeng et al. (2008) observed increased litter size in response to Arg throughout pregnancy and a greater number of surviving embryos. Zeng et al. (2008) stated that these results indicate that dietary Arg supplementation enhances embryonic survival; therefore, increasing litter size by 30% at term birth and supports implications for enhancing reproductive performance in mammals.

Injectable or Dietary Alanine

Many of the previous trials have used injectable or dietary alanine as an isonitrogenous control for Arg treatments (Mateo et al., 2007 and 2008; Zeng et al., 2008). Although plasma concentrations of alanine were increased in the supplemented animals in all trials, the

reproductive performance was similar to that of the non-supplemented animals; therefore alanine had no adverse or beneficial effect on reproductive performance (Mateo et al., 2007).

Rumen-Protected Amino Acids

An interest in ruminally-protected amino acids began with the identification of Lys and Met as two of the most limiting amino acids for protein synthesis in ruminants (NRC, 2001). One of the main challenges in diet formulation, particularly for animals requiring higher RUP diets, is to achieve the desired concentrations of essential amino acids in MP by relying solely on feed protein requirements (NRC, 2001). Technologies are currently being developed to allow amino acids, such as Met and Lys, to escape ruminal degradation without compromising their digestibility in the small intestine.

Methods that have been evaluated for protecting free amino acids from ruminal degradation have been reviewed (Loerch and Oke, 1989; Schwab, 1995). Currently, there are two categories of technology for ruminally protected amino acids: (1) surface coating with a fatty acid/pH-sensitive polymer mixture, or (2) surface coating or matrices involving fat or saturated fatty acids and minerals. The first technological approach of rumen-protected amino acids has the best bioavailability (NRC, 2001).

Rumen-Protected Arginine

Peine et al. (2013) conducted a study evaluating the effects of rumen-protected Arg supplementation during gestation in ewes on postnatal offspring performance. The ewes were first confirmed pregnant and assigned to one of three treatments: control, restricted, and restricted with a rumen-protected arginine supplement. On d 54 of gestation, control ewes were fed a diet formulated to meet 100% NRC requirements, restricted ewes were fed 60% of controls, and the Arg supplemented group received a granular Arg supplement at 180 mg/kg BW

daily. Rumen protected Arg supplements were mixed with 49.9 g of corn and fed once a day. At lambing, lambs were not permitted to nurse and administered artificial colostrum and then provided milk replacer and creep. Body weights of lambs from restricted and restricted with Arg supplement ewes were similar at birth, weighing less than lambs from control ewes. By d 19, lambs from restricted with Arg supplement ewes weighed more than lambs from restricted ewes, and were similar to weights of lambs from control ewes.

Saevre et al. (2011b) studied the effects rumen-protected Arg supplementation from d 8 to d 13 of the estrous cycle on ewe serum-amino-acid concentration, circulating progesterone, and ovarian blood flow. Ewes were placed on one of four treatments: 0 (CON), 90 mg/kg BW supplemental Arg (90 ARG), 180 mg/kg BW supplemental Arg (180 ARG) and 360 mg/kg BW supplemental Arg (360 ARG). Ewes were individually fed their respective treatment blended with 150 g ground corn, which was immediately followed by 650 g of a pelleted diet. Ewes that were fed 360 ARG generally had greater serum-Arg concentrations than CON, 90 ARG, and 180 ARG on d 11 and d 12. On d 11, arginine as a percent of total amino acid concentration was greater in 360 ARG compared with CON and 90 ARG. Total essential amino-acid concentration was elevated in 360 ARG compared with 90 ARG and 180 ARG on d 12. Arginine supplementation increased peak systolic velocity in the corpus luteum (CL) for 360 ARG and 90 ARG compared to CON. Supplemental rumen-protected Arg had no effect on serum concentration of progesterone. This trial indicated that when rumen-protected Arg is supplemented to ewes at the rate of 360 mg/kg BW might increase circulating serum Arg concentration, in addition to increasing ovarian blood flow.

Feedstuff Composition and Degradability

In addition to "artificially" protecting amino acids from ruminal degradation through a coating, "naturally" occurring bypass proteins may provide a source of Arg to the small intestine of ruminants. Hussein and Jordan (1991) have investigated feeding fishmeal compared to other protein sources to determine the extent of ruminal protein degradation, amount of feed protein available for ruminal microbes, and intestinal digestion by the host animal. Hussein and Jordan (1991) reported that *in situ*, FM had a slower rate of digestion and N digestion when compared to SBM and corn. Davenport et al. (1990) concluded that inclusion of fishmeal in a diet of ground soybean meal increased plasma concentrations of arginine along with related amino acids, ornithine, and citrulline with 70% of protein in fishmeal escaping ruminal degradation (Mercer et al., 1980). Tamminga (1982) stated that fishmeal is a valuable source of protein for growing ruminants due to its high protein content, ruminal stability, and amino acid profile. O'Mara et al. (1997) investigated amino acid composition of many feedstuffs including fishmeal and soybean meal, observing that ruminant feeding systems are constrained by limited knowledge of the amino acids absorbed from the small intestine and the amino acid profile of the absorbed fraction of bypass protein can be different from the original feed, which could make predictions of absorbed amino acids difficult. O'Mara et al. (1997) reported that fishmeal samples had between 72.9 and 83.6% amino acid nitrogen while soybean meal and sopralin samples had similar amino acid nitrogen with 76 and 73%, respectively. O'Mara et al. (1997) also indicated that sopralin had no differences in the amino acid profile before or after 8 or 12 h of ruminal incubation and was also minimal in fishmeal samples. It was also reported that Arg was one of the more susceptible amino acids to ruminal degradation and was lower in proportion in all the feedstuff residues than in the original feedstuffs (O'Mara et al., 1997). Interestingly, O'Mara et al. (1997)

observed that Arg had increased disappearance postruminally following 12 h of ruminal incubation in fishmeal samples and the sopralin sample (27.3, 25.3, and 22.4 g/kg DM of original feedstuff; respectively) when compared to soybean meal, rapeseed meal, cottonseed meal, corn distillers grain, and corn gluten feed (10.6, 4.9, 13.3, 4.6, 1.4 g/kg DM of original feedstuff; respectively). Although more research needs to be done to elucidate RUP and MP along with amino acid composition of feeds, the previous results are very useful for applying to the current research. Fishmeal withstood ruminal incubation to a greater extent than soybean meal not treated with formaldehyde, allowing for more amino acids, including Arg, to be absorbed and utilized in the small intestine.

Conclusion

Results from previous research indicate Arg supplementation can have many positive effects on reproductive efficiency in ewes, especially when in a compromised status, and lamb performance. However, previous research has almost always occurred in a compromised situation for the ewe, in most cases on a restricted nutrient intake. In other cases, out of season breeding with estrus induced via hormones was utilized. Arginine supplementation increased birth weights of lambs (Lassala et al., 2010), gain in lambs (Peine et al., 2013), and peak systolic velocity to the CL (Saevre et al., 2011b). Recent trials have utilized a rumen-protected arginine product, making it feasible for producers to replicate these results (Saevre et al., 2011b). Further research needs to be done with both injectable Arg and rumen-protected products (both artificially coated and natural rumen bypass) to indicate if Arg supplementation only has effects in compromised situations, such as underfed or hormonally stimulated ewes. Synthetic and the naturally protected forms of Arg need to be investigated further to find out if replication of the results of the injectable Arg trials is possible. Another area that could be investigated is the

possibility of NO toxicity due to supplementation of Arg, looking at the negative effects on lambs postnatally to weaning and beyond.

Literature Cited

Ball, R. O., K. L. Urschel, and P. B. Pencharz. 2007. Nutritional consequences of interspecies differences in arginine and lysine metabolism. J. Nutr. 137(6):1626S-1641S.

Barrell, G. K., L. A. Thrun, M. E. Brown, C. Viguie, and F. J. Karsch. 2000. Importance of photoperiodic signal quality to entrainment of the circannual reproductive rhythm of the ewe. Biol. Reprod. 63:769-774.

Bazer, F. W., R.M. Roberts, and W.W. Thatcher. 1979. Actions of hormones on the uterus ad effect on conceptus development. J. Anim. Sci. 49:35-45.

Bazer, F. W., T. L. Ott, and T. E. Spencer. 1998. Maternal recognition of pregnancy: comparative aspects. Tropho. Res. 12:375-386.

Bloomfield, F. H. 2011. Epigenetic modifications may play a role in the developmental consequences of early life events. J. Neurodev. Disord. 3(4):348-355.

Bolet, G. 1986. Timing and extent of embryonic mortality in pigs, sheep and goats: genetic variability. In: Sreenan J.M., Diskin, M. G., editors. *Embryonic mortality in farm animals*. Boston: Kluwer Academic Publishing; 1986:12.

Bradford, G. E. and H. H. Meyer. 1986. Economic evaluation of breeding objectives for sheep and goats: practical considerations and examples. 3rd world congress on genetics applied to livestock production. Paper 20.

Burton, G. J., A. I. Watson, J. Hempstock, J. N. Skepper, and E. Jauniaux. 2002. Uterine glands provide histotrophic nutrition for the human fetus during the first trimester of pregnancy. J. Clinical Endo. and Met. 87:2954-1959.

Carson, D. D., I. Bagchi, S. K. Dey, A.C. Enders, A. T. Fazlebas, B. A. Lessey and K. Yoshinaga. 2000. Embryo implantation. Developmental Bio. 223:217-237.

Chwalisz, K., E. Winterhager, T. Thienel, and R. E. Garfield. 1999. Synergistic role of nitric oxide and progesterone during the establishment of pregnancy in the rat. Hum. Reprod. 14:542-552.

Cross, J. C. 2001. Genes regulating embryonic and fetal survival. Therio. 55:193-207.

Davenport, G. M., J. A. Boling, and N. Gay. 1990. Performance and plasma amino acids of growing calves fed corn silage supplemented with ground soybeans, fishmeal and rumen-protected lysine. J. Anim. Sci. 68:3773-3779.

De Boo, H. A., P.L. van Zijl, D. E. Smith, W. Kulik, H. N. Lafeber, and J.E. Harding. 2005. Arginine and mixed amino acids increase protein accretion in the growth-restricted and normal ovine fetus by different mechanisms. Pediatr. Res. 58:270-277.

Dixon, A. B., M. Knights, J. L. Winkler, D. J. Marsh, J. L. Pate, M. E. Wilson, R. A. Dailey, G. Seidel, and E. K. Inskeep. 2007. Patterns of late embryonic and fetal mortality and association with several factors in sheep. J. Anim. Sci. 85:1274-1284.

Flechon, J. E., M. Guilomot, M. Chrlier, B. Flechon, and J. Martal. 1986. Experimental studies on the elongation of the ewe blastocyst. Repro. Nutr. Dev. 26:1017-1024.

Flynn, N. E., C. J. Meininger, T. E. Haynes, and G. Wu. 2002. The metabolic basis of arginine nutrition and pharmacotherapy. Bio. and Pharm. 56(9):427-438.

Gouge, R. C., P. Marshburn, B.E. Gordon, W. Nunley, and Y. M. Huet-Hudson. 1998. Nitric oxide as a regulator of embryonic development. Biol. Reprod. 58:875-879.

Gray, C. A., F. F. Bartol, B. J. Tarleton, A. A. Wiley, G. A. Johnson, F. W. Bazer, and T. E. Spencer. 2001a. Developmental biology of uterine glands. Biol. Reprod. 65:1311-1323.

Gray, C. A., K. M. Taylor, W. S. Ramsey, J. R. Hill, F. W. Bazer, F. F. Bartol, and T. E. Spencer. 2001b. Endometrial glands are required for preimplantation conceptus elongation and survival. Biol. Repro. 64: 1608-1613.

Gray, C. A., R. C. Burghardt, G. A. Johnson, F. W. Bazer, and T. E. Spencer. 2002. Evidence that absence of endometrial gland secertions in uterine gland knockout ewes compromises conceptus survival and elongation. J. Reprod. 124: 289-300.

Greenberg, S. S., J. R. Lancaster, J. Xie, T. G. Sarphie, X. Zhao, L. Hua, T. Freeman, D. R. Kapusta, T. D. Giles, and D. R. Powers. 1997. Effects of NO synthase inhibitors, arginine-deficient diet, and amiloride in pregnant rats. Am. J. Physiol. Regul. Integr. Comp. Physiol. 273:R1031-R1045.

Hulet, C. V., H. P. Voigtlander, A. L. Pope, and L. E. Casida. 1956. The nature of early season infertility in sheep. J. Anim. Sci. 15:607-616.

Hussein, H. S. and R. M. Jordan. 1991. Fish meal as a protein supplement in finishing lamb diets. J. Anim. Sci. 69:2115-2122.

Ignarro, L. J., G. M. Buga, K. S. Wood, R. E. Byrns, and G. Chaudhuri. 1987. Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. Proc. Natl. Acad. Sci. 84:9265-9269.

Karsch, F. J., J. E. Robinson, C. J. Woodfill, and M. B. Brown. 1989. Circannual cycles of luteinizing hormone and prolactin secretion in ewes during prolonged exposure to a fixed photoperiod: evidence for an endogenous reproductive rhythm. Biol. Repro. 41:1034-1046.

Knights, M., Q. S. Baptiste, A. B. Dixon, J. L. Pate, D. J. Marsh, E. K. Inskeep, and P. E. Lewis. 2003. Effects of dosage of FSH, vehicle and time of treatment on ovulation rate and prolificacy in ewes during the anestrous season. Small Rum. Res. 50:1-9.

Kwon, H., T. E. Spencer, F. W. Bazer, and G. Wu. 2003a. Developmental changes of amino acids in ovine fetal fluids. Biol. Reprod. 68:1813-1820.

Kwon, H., G. Wu, F. W. Bazer, and T. E. Spencer. 2003b. Developmental changes in polyamine levels and synthesis in the ovine conceptus. Biol. Reprod. 69:1626-1634.

Kwon, H., G. Wu, C. J. Meininger, F. W. Bazer, and T. E. Spencer. 2004. Developmental changes in nitric oxide synthesis in the ovine placenta. Biol. Reprod. 70:679-686.

Lawson, R. A., R. A. Parr, and L. P. Cahill. 1983. Evidence for maternal control of blastocyst growth after asynchronous transfer of embryos to the uterus of the ewe. J. Reprod. and Fert. 67:477-483.

Lassala, A., F. W. Bazer, T. A. Cudd, S. Datta, D. H. Keisler, M. C. Satterfield, T. E. Spencer, and G. Wu. 2010. Parenteral administration of L-arginine prevents fetal growth restriction in undernourished ewes. J. Nutr. 140:1242-1248.

Lassala, A., F. W. Bazer, T. A. Cudd, S. Datta, D. H. Keisler, M. C. Satterfield, T. E. Spencer, and G. Wu. 2011. Parenteral administration of L-arginine enhances fetal survival and growth in sheep carrying multiple fetuses. J. Nutr. 141:849-855.

Loerch, S. G., and Oke, B. O. 1989. Rumen-protected amino acids in ruminant nutrition. *In* Absorption and Utilization of Amino Acids. 3:187-200. CRC Press, Boca Raton, FL.

Lupton, C. J. 1998. ASAS CENTENNIAL PAPER: Impacts of animal science research on United States sheep production and predictions for the future. J. Anim. Sci. 86:3253-3274.

Luther, J. S., E. J. Windorski, J. S. Caton, G. Wu, J. D. Kirsch, K. A. Vonnahme, L. P. Reynolds, and C. S. Schauer. 2009. Effects of arginine supplementation on reproductive performance in Rambouillet ewes. NDSU Sheep Res. Rep. 50:11-13.

Malpaux, B., C. Viguie, D. C. Skinner, J. C. Thiery, J. Pelletier, and P. Chemineau. 1996. Seasonal breeding in sheep: Mechanism of action of melatonin. Anim. Repro. Sci. 42:109-117.

Manser, R. C., H. J. Leese, and F. D. Houghton. 2004. Effect of inhibiting nitric oxide production on mouse perimplantation embryo development and metabolism. Biol. Repro. 71:528-533.

Mateo, R. D., G. Wu, F. W. Bazer, J. C. Park, I. Shinzaro, and S. W. Kim. 2007. Dietary L-arginine supplementation enhances the reproductive performance of gilts. J. Nutr. 137:652-656.

Mateo, R. D., G. Wu, H. K. Moon, J. A. Carroll, and S. W. Kim. 2008. Effects of dietary arginine supplementation during gestation and lactation on the performance of lactating primiparous sows and nursing piglets. J. Anim. Sci. 86:827-835.

Mercer, J. R., S. A. Allen, and E. L. Miller. 1980. Rumen bacterial protein synthesis and the proportion of dietary protein escaping degradation of sheep. Br. J. Nutr. 43:421-433.

Moncada, S. and E. A. Higgs. 1993. The L-arginine-nitric oxide pathway. N. Engl. J. Med. 392:2002-2012.

Moore, N. W., L. E. Rowson, and R. V. Short. 1960. Egg transfer in sheep: Factors affecting the survival and development of the transferred eggs. J. Repro. and Fertil. 1:322-349.

NASS. 2014. National Agriculture Statistics Service. Available: http://www.nass.usda.gov/index.asp. Accessed: Feb. 12, 2014.

Nephew, K. P., K. E. McClure, and W. F. Pople. 1989. Embryonic migration relative to maternal recognition of the pregnancy in sheep. J. Anim. Sci. 69:999-1005.

Nephew, K. P., K. E. McClure, T. L. Ott, D. H. Dubois, F. W. Bazer, and E. F. Pope. 1991. Relationship between variation in conceptus development and differences in estrous cycle duration in ewes. Biol. Repro. 44:536-539.

NRC. 2001. Nutrient requirements of dairy cattle. Natl Acad Press, Washington, DC.

O'Mara, F. P., J. J. Murhy, and M. Rath. 1997. The amino acid composition of protein feedstuffs before and after ruminal incubation and after subsequent passage through the intestines of dairy cows. J. Anim. Sci. 75:1941-1949.

Osawska, S., C. Moinard, C. Loi, N. Neveux, and L. Cynober. 2004. Citrulline increases arginine pools and restores nitrogen balance after massive intestinal resection. Gut. 53(12):1781-1786.

Parr, R. A. 1992. Nutrition-progesterone interactions during early pregnancy in sheep. J. Repro. and Fert. 4(3):297–300.

Peine, J. L., G. Q. Jia, M. L. Van Emon, T. L. Neville, J. D. Kirsch, C. J. Hammer, L. P. Reynolds, and J. S. Caton. 2013. Effects of maternal nutrition and rumen-protected arginine supplementation on ewe and postnatal lamb performance. In: Western Sect. ASAS Proc., Bozeman, MT. 64:80-83.

Quinlivan, T. D. 1966. Estimates of pre- and perinatal mortality in the New Zealand Romney Marsh ewe. J. Reprod. Fertil. 11:379-390.

Rhind, S. M., J. J. Robinson, and I. McDonald. 1980. Relationships among uterine and placental factors in prolific ewes and their relevance to variations in fetal weight. Anim. Prod. 30:115-124.

Roberts, R. M. and F. W. Bazer. 1988. The functions of uterine secretions. J. Repro. and Fert. 82:875-892.

Robinson, J. J., A. J. F. Russel, T. T. Treacher, and J. B. Kilkenny. 1983. Feeding the ewe. Milton Keynes, UK: Meat and Livestock Commission Sheep Improvement Services. 1983. 1-60.

Rosa, H. J. D. and M. J. Bryant. 2003. Seasonality of reproduction in sheep. Small Rum. Res. 48:155-171.

Saevre, C., A. M. Meyer, M. L. Van Emon, D. A. Redmer, J. S. Caton, J. D. Kirsch, J. S. Luther, and C. S. Schauer. 2011a. Impacts of arginine on ovarian function and reproductive performance at the time of maternal recognition of pregnancy in ewes. NDSU Sheep Res. Rep. 52:13-16.

Saevre, C. B., J. S. Caton, J. S. Luther, A. M. Meyer, D. V. Dhuyvetter, R. E. Musser, J. D. Kirsch, M. Kapphahn, D. A. Redmer, and C. S. Schauer. 2011b. Effects of rumen-protected arginine supplementation on ewe serum-amino-acid concentration, circulating progesterone, and ovarian blood flow. Sheep and Goat Res. J. 26:8-12.

Schillo, K. K. 2009. Reproductive Physiology of Mammals: From farm to field and beyond. 1st ed. Delmar, Clifton Park, NY.

Schrick, F. N. and E. K. Inskeep. 1993. Determination of early pregnancy in ewes utilizing transrectal ultrasonography. Therio. 40:295-306.

Schwab, C. G. 1995. Protected proteins and amino acids for ruminants. Biotechnology in Animal Feeds and Animal Feeding. V.C.H. Press, Weinheim, Germany: 115-141.

Self, J. T., T. E. Spencer, G. A. Johnson, J. Hu, F. W. Bazer, and G. Wu. 2004. Glutamine synthesis in the developing porcine placenta. Biol. Reprod. 70:1444-1451.

Senger, P. L. 2005. Pathways to pregnancy and parturition. 2nd Ed. Pullman, WA: Current Conceptions.

Spencer, T. E. and F. W. Bazer. 2002. Biology of progesterone action during pregnancy recognition and maintenance of pregnancy. Front. Biosci. 7:1879-1898.

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

Tamminga, S. 1982. Energy-protein relationships in ruminant feeding: Similarities and differences between rumen fermentation and postruminal utilization. In: E. L. Miller, I. H. Pike, and A. J. H. Van Es (Ed.) Protein Contribution of Feedstuffs for Ruminants: Application to Feed Formulation. pp 4-17. Butterworth Scientific, London.

Thureen, P. J., K A. Baron, P. V. Fennessey, and W. W. Hay Jr. 2002. Ovine placental and fetal arginine metabolism at normal and increased maternal plasma arginine concentrations. Pediatr. Res. 51:464-471.

Vivien-Roels, B. and Pévet, P. 1983. The pineal gland and the synchronization of reproductive cycles with variations of the environmental climatic conditions, with special reference to temperature. Pineal Res. Rev. 1: 91–143.

Vosatka, R. J., P. M. Hassoun, and K. B. Harvey Wilkes. 1998. Dietary l-arginine prefects fetal growth restriction in rats. Am. J. Obstet. Gyn. 178:242-246.

Wilmut, I., D. I. Sales, and C. J. Ashworth. 1986. Maternal and embryonic factors associated with prenatal loss in mammals. J. Repro. Fert. 76(2):851-864.

Windmueller, H. G. and A. E. Spaeth. 1981. Source and fate of circulating citrulline. Am. J. Physiol. 241:E473-E480.

Woodfill, C. J., J. E. Robinson, B. Malpaux, and F. J. Karsch. 1991. Synchronization of the circannual reproductive rhythm of the ewe by discrete photoperiodic signals. Biol. Repro. 45:110-121.

Wu, G. and D. A. Knabe. 1995. Arginine synthesis in enterocytes of neonatal pigs. Am. J. Physiol. 269:R621-R629.

Wu, G. 1997. Synthesis of citrulline and arginine from proline in enterocytes of postnatal pigs. Am. J. Physiol. 272:G1382-G1390.

Wu, G., P. K. Davis, N.E. Flynn, D. A. Knabe, and J. T. Davidson. 1997. Endogenous synthesis of arginine plays an important role in maintaining arginine homeostasis in postweaning growing pigs. J. Nutr. 127:2342- 2349.

Wu, G. and S. M. Morris. 1998. Arginine metabolism: nitric oxide and beyond. Biochem. J. 336:1-17.

Wu, D., W. G. Pond, T. Ott, and F. W. Bazer. 1998. Maternal dietary protein deficiency decreases amino acid concentrations in fetal plasma and allantoic fluid pigs. J. Nutr. 128:894-902.

Wu, G., F. W. Bazer, T. A. Cudd, C. J. Meininger, and T. E. Spencer. 2004. Maternal nutrition and fetal development. J. Nutr. 134:1249-1252.

Wu, G. F. W. Bazer, J. Hu, G. A. Johnson, and T. E. Spencer. 2005. Polyamine synthesis from proline in the developing porcine placenta. Biol. Reprod. 72:842-850.

Wu, G., F. W. Bazer, J. M. Wallace, and T. E. Spencer. 2006. BOARD-INVITED REVIEW: Intrauterine growth retardation: Implications for the animal sciences. J. Anim. Sci. 84:2316-2337.

Wu, G., F. W. Bazer, T. A. Davis, S. W. Kim, P. Li, J. M. Rhoads, M. C. Satterfield, S. B. Smith, T. E. Spencer, and Y. Yin. 2009. Arginine metabolism and nutrition in growth, health and disease. Amino Acids. 37:153-168.

Zeng, X., F. Wang, X. Fan, W. Yang, B. Zhou, P. Li, Y. Yin, G. Wu, and J. Wang. 2008. Dietary arginine supplementation during early pregnancy enhances embryonic survival in rats. J Nutr. 138:1304-1309.

CHAPTER 2. IMPACTS OF SUPPLEMENTAL ARGININE ON EWE REPRODUCTIVE PERFORMANCE

Abstract

In sheep, embryonic and fetal death during pregnancy can account for 25 to 50% of the total number of ovulations. The objective of this study was to determine the effects of injectable and oral arginine (Arg) supplementation provided for 14 d post-breeding on reproductive performance of naturally stimulated fall lambing ewes. Two hundred ten Rambouillet ewes were exposed to rams equipped with marking harnesses to induce cyclicity. Upon estrus detection (d 0) ewes were randomly assigned to one of six treatments for a 14-d treatment period: injectable saline **(CON**; n = 25), injectable alanine (**IVALA**; n = 20), injectable arginine (**IVARG**; n = 23), oral rumen-protected Arg (**RPARG**; n = 20), oral soybean meal (**SBM**; n = 23), or oral fishmeal (**FM**; n = 24). Daily treatments, except CON, ALA, and SBM, were formulated to provide supplemental Arg at approximately 30 mg/kg ewe BW. Ewes receiving injectable treatments were provided 454 g/d corn post-injection. Oral supplements were ground and provided individually to ewes at 0800 daily. Serum/plasma samples were collected on d 0, 2, 4, 6, 8, 10, 12, and 14 from 12 ewes per treatment to evaluate serum progesterone and amino acid concentrations. At lambing, birth weight, birth type, and sex were recorded. Weaning weights were recorded when the average age of lambs was 85 d. No differences ($P \geq 0.06$) were detected for pregnancy and lambing rates or birth and lamb weaning weights among treatments. However, litter weaning weight tended ($P = 0.06$) and weaning rates were higher ($P = 0.05$) in Arg supplemented ewes. Plasma progesterone and serum Arg concentrations showed a treatment and day effect ($P < 0.0001$), but no treatment × day interaction ($P \geq 0.99$) was observed. In contrast

to previous research, supplemental Arg during the first 14 d of pregnancy did not improve pregnancy or lambing rates, however, IVARG did positively impact weaning rates.

Key Words: arginine, progesterone, reproduction, sheep

Introduction

The majority of embryonic loss in sheep occurs before d 18 of gestation (Hulet et al., 1956; Moore et al., 1960; Quinlivan, 1966). Loss of individual embryos can occur without a complete loss of pregnancy, such as in the case of multiple fetuses (Rhind et al., 1980; Schrick and Inskeep, 1993). In sheep, it has been reported that 30% of fertilized ova are not represented by live births, resulting in frequent, but unrecognized loss (Bolet, 1986; Knights et al., 2003; Dixon et al., 2007). Current research has indicated periconceptual supplementation of Arg can recover these losses (Luther et al., 2009; Saevre et al., 2011a).

Treatment with the amino acid Arg in late pregnancy increases transport of nutrients to the unborn lamb through the synthesis of polyamines and NO as observed by Thureen (2002), along with enhancements in fetal protein levels in sheep and increases in lamb birth weight (De Boo, 2005). Treatment of ewes with injectable Arg during maternal recognition of pregnancy improved pregnancy rate by 24% (Saevre et al., 2011a) and when given for 15 d post-breeding, increased lambing rate by 50% (Luther et al., 2009). Previous research utilized an injectable Arg source, and for these results to become commercially applicable an oral form of supplemental Arg has to be developed. The objective of this study was to determine the effects of injectable and oral Arg supplementation provided 2 wk post-breeding on reproductive performance of naturally stimulated fall lambing ewes. Our hypothesis was injectable and supplemental Arg would increase pregnancy and lambing rates while decreasing postnatal lamb loss.

Materials and Methods

All procedures were approved by the Animal Care and Use Committee of North Dakota State University (Protocol #A12038). This study was conducted at the Hettinger Research Extension Center in Hettinger, ND.

Animals and Diets

Two hundred ten multiparous Rambouillet ewes (64.7 ± 6.8 kg BW) were supplemented with 454 g/d of corn and exposed to 15 ram lambs to induce estrus in April of 2012. Ram lambs equipped with marking harnesses were introduced to ewes 12 d prior to the initiation of the study. Three ewes that were marked by ram lambs prior to study initiation were removed from the trial. Mature Rambouillet rams (n = 10) equipped with marking harnesses were introduced to the ewe flock on d 0. Ewes were monitored daily at 0800 to determine onset of estrus as indicated by breeding marks from harnessed mature rams. Once estrus was detected, ewes were randomly assigned to one of six treatment groups: control (**CON**; n = 25), IV-alanine (**IVALA**; n = 20; Ajinomoto North America, Inc., Raleigh, NC), IV-arginine (**IVARG**; n = 23; Ajinomoto North America, Inc., Raleigh, NC), rumen-protected arginine (**RPARG**; n = 20), soybean meal (**SBM**; n = 23), and fishmeal (**FM**; n = 24) and received the treatments indoors at an ambient temperature of 22.2 ± 6 ° C. Ewes assigned to IVARG, IVALA, and CON were administered intravenous injections of Arg (30 mg • kg BW^{-1} • d^{-1} of l-arginine), alanine (Ala; 30 mg • kg BW^{-1} • d^{-1} of l-alanine), and saline daily, respectively. All ewes were injected with similar treatment volumes (0.100 mL • kg BW^{-1} • d^{-1}) of saline or their respective amino acid.

All ewes receiving injectable treatments were individually supplemented 454 g of corn daily. Similar to Luther et al. (2009) and Saevre et al. (2011a), the IVARG and IVALA treatments were designed to provide 30 mg • kg BW^{-1} • d^{-1} of metabolically available Arg or Ala;

respectively. Oral supplementation treatments included: RPARG (0.15 g/kg BW of rumen protected product mixed with ground corn), SBM (37.5:62.5 soybean meal:corn), and FM (25:75 fishmeal:corn). All ewes were individually supplemented 454 g/d of their respective treatments.

The RPARG and FM treatments were designed to provide 30 mg • kg BW^{-1} • d^{-1} of metabolically available Arg, whereas SBM was designed to be isonitrogenous to FM. During the entire treatment period, ewes were group fed a basal ration daily consisting of 1.88 kg alfalfa hay, 0.226 kg of barley haylage, and 0.023 kg trace mineral (DM basis; Table 2.1). Ewes were provided water ad libitum.

Table 2.1 describes the nutrient concentration of the dietary ingredients, including Arg %, rumen undegradable Arg (**RUArg**), microbial Arg (**MicrArg**), and metabolically available Arg (**MetArg**). From d 0 (estrus) until d 14, ewes individually received their assigned treatment. All treatments were imposed within 15 d of mature ram introduction. Ewes that were removed from the trial were either bred on the second cycle or died from non-treatment related problems. Feed samples from the basal ration were taken once every 2 d as bunks were filled. Samples of treatment feeds were taken each time a new ration was mixed. Common samples were compiled together, mixed and sent to University of Missouri, Agricultural Experiment Station Chemical Laboratories for analysis of Arg concentrations by AOAC Official Method 982.30 E (AOAC Int., 2000) similar to Brooks et al. (2011). Diets were collected and sent to Midwest Laboratories, Inc., Omaha, NE for analysis of DM (method 930.15; AOAC Int., 1990), ash (method 942.05; AOAC Int., 2010), CP (method 2001.11; AOAC Int., 2010) using a Kjeltec Auto 1030 Analyzer, and ADF (method 973.18; AOAC Int., 1990).

Arginine was provided via the basal ration and through the feeds each treatment received (g/d; Table 2.2). Rumen undegradable Arg (**RUArg**), microbial Arg (**MicrArg**), and total Arg

(**Total Arg**) provided to the ewe for absorption each day in mg • kg BW^{-1} • d^{-1} is also provided in Table 2.2. CON and IVALA treatments received 203.13 mg • kg BW^{-1} • d^{-1} and the IVARG ewes receiving 233.13 mg • kg BW^{-1} • d^{-1} of Total Arg. RPARG treated ewes received a total of 250 mg • kg BW^{-1} • d^{-1} Arg, as did the SBM treated ewes. Ewes supplemented with FM were receiving a total of 281.25 mg • kg BW^{-1} • d^{-1} Arg. Each Arg supplement treatment received at least the target of 30 mg • kg BW^{-1} • d^{-1} when compared to the CON. However, the SBM treatment was receiving an equal amount of Arg to the RPARG treated ewes and FM receiving the greatest supplementation of Arg.

Table 2.1. Nutrient composition of feed ingredients and supplements of basal ration[1]

	Diet Ingredients and Nutrient Concentration[2]					
Item[3]	Alfalfa Hay	Barley Haylage	Corn	FM	SBM	RPArg
Diet, % DM	92.0	33.0	88.0	90.0	91.0	-
CP, % DM	18.1	12.4	9.0	64.2	46.5	-
ADF, % DM	43.2	31.6	3.0	2.0	10.0	-
TDN, %DM	53.3	66.5	88.0	74.0	84.0	-
Ca, % DM	1.75	0.39	0.02	5.5	0.38	-
P, % DM	0.22	0.38	0.3	3.15	0.71	-
Cu, ppm	0.008	0.006	0.0004	0.0011	0.0025	-
Arg, %	0.42	0.16	0.38	3.6	3.3	60.0
RUArg, %	0.13	0.04	0.21	2.5	1.3	30.0
MicrArg, %	0.35	0.43	0.57	2.4	1.2	-
MetArg, %	0.47	0.47	0.78	5.0	2.5	-

[1]Ad libitum diet provided: 90% alfalfa hay and 10% barley haylage offered (DM basis); trace mineral content: 16-18% Ca; 8% P; 12-14% salt; 2.5% Mg; 4% Co; 100 ppm I; 1,800 ppm Mn; 20 ppm Se; 2,000 ppm Zn; 113,500 IU/kg Vitamin A; 11,350 IU/kg Vitamin D; and 227 IU/kg Vitamin E.

[2]Corn= includes CON (IV saline), IVALA (30 mg • kg BW^{-1} • d^{-1} l-alanine), and IVARG (30 mg • kg BW^{-1} • d^{-1} l-arginine); RPARG = 454 g/d corn and 0.15 g • kg BW^{-1} • d^{-1} rumen-protected Arg; FM = 454 g/d 25:75 FM: corn; SBM = 454 g/d 37.5:62.5 SBM: corn.

[3]RUArg= rumen undegradable Arg (%Arg*RUP); MicrArg= microbial Arg [(TDN*.13)*%Arg]; MetArg= metabolically available Arg (RUArg + MicrArg), calculated from NRC (2007).

Table 2.2. Nutrients and Arginine concentration supplied from treatment diets and basal ration[1]

	Diet Ingredients and Arginine Concentration (DM basis)[2]					
Item	CON	IVALA	IVARG	RPARG	SBM	FM
Diet, g/d	2,505	2,505	2,505	2,509	2,506	2,515
CP	404	404	404	404	462	460
ADF	896	896	896	896	906	895
TDN	1504	1504	1504	1504	1500	1491
Ca	34	34	34	34	34	39
P	6	6	6	6	6	9
Cu, ppm	0.02	0.02	0.02	0.02	0.02	0.02
Total Arg[3], mg • kg BW^{-1} • d^{-1}	203	203	233	250	250	282
Arg[4]	156	156	156	250	219	203
RUArg[4]	47	47	77	94	78	94
MicrArg[5]	156	156	156	156	172	188

[1]CON= IV saline; IVALA= 30 mg • kg BW^{-1} • d^{-1} l-alanine; IVARG= 30 mg • kg BW^{-1} • d^{-1} l-arginine; RPARG= 454 g/d corn and 0.15 g • kg BW^{-1} • d^{-1} rumen-protected Arg; FM = 454 g/d 25:75 FM: corn; SBM = 454 g/d 37.5:62.5 SBM: corn.
[2]SBM and FM ingredients calculated based on O'Mara et al. (1997) values; ground corn calculation (component of each treatment) based on NRC (2007).
[3]Total Arg= total Arg provided for absorption (RUArg + MicrArg).
[4]Arg= total Arg provided by the diet.
[4]RUArg= rumen undegradable Arg (%Arg*RUP), calculated from NRC (2007).
[5]MicrArg= microbial Arg [(TDN*0.13)*%Arg)], calculated from NRC (2007).

Ewes were allowed to graze and checked daily at approximately d 55 of gestation. At approximately 130 d of pregnancy, ewes were brought to the Hettinger Research Extension Center lambing barn and bunk fed in pens of 15 a total mixed ration of 0.242 kg ground hay, 0.607 kg barley grain, 0.773 kg barley haylage, and 0.023 kg of trace mineral (DM basis; Table 2.3). Lambs were fed a grower pellet ad libitum which was 38% crude protein, 1% crude fat, 4-5% Ca, 0.6% P, 3.5-4.5% salt, 3.0 ppm Se, 52,800 IU/kg Vitamin A, 5280 IU/kg Vitamin D, and 132 IU/kg Vitamin E. The grower pellet was also medicated with chlortetracycline at a rate of 4.4 g/kg of feed. Lambs were weaned at approximately 85 d of age.

Table 2.3. Nutrient composition of ration ingredients in late gestation and lactation[1]

	Diet Ingredients and Nutrient Concentration[2]			
Item	Ground Hay	Barley Haylage	Barley Grain	Total
Diet, % DM	85.9	26.9	89.2	95.7
CP, % DM	15.0	18.3	13.6	26.0
ADF, % DM	39.6	28.2	6.2	35.2
TDN, %DM	63.1	68.7	87.2	121.0
Ca, % DM	1.38	0.80	0.08	1.4
P, % DM	0.18	0.36	0.50	0.81
Cu, ppm	6.0	5.0	8.0	19.0

[1]Ad libitum diet 0.242 kg ground hay, 0.773 kg barley haylage, 0.607 kg barley grain, and 0.023 kg of trace mineral (DM basis).

[2]Trace mineral content: 16-18% Ca; 8% P; 12-14% salt; 2.5% Mg; 4% Co; 100 ppm I; 1,800 ppm Mn; 20 ppm Se; 2,000 ppm Zn; 113,500 IU/kg Vitamin A; 11,350 IU/kg Vitamin D; and 227 IU/kg Vitamin E.

Sample collection

Blood samples were collected via jugular venipuncture using a 20 G × 2.54 cm needle into 10 mL serum/plasma tubes (BD Vacutainer Serum, Becton, Dickinson and Company, Franklin Lakes, NJ; BD Short Bevel Needles, #305178, Becton, Dickinson and Company, Franklin Lakes, NJ) on d 0, 2, 4, 6, 8, 10, 12, and 14 from 12 random ewes per treatment prior to administration of treatment at 0800 and immediately placed on ice. Samples were then centrifuged at 4° C for 30 min at 1500 × g and serum/plasma was transferred into plastic 2.0 mL mirocentrifuge tubes and frozen at -20° C until assayed. Serum samples were assayed for concentrations of circulating progesterone using IMMULITE 1000 (Siemens Healthcare Diagnostics; Galbreath et al., 2008). Serum samples were assayed (n=5) for amino acid concentrations similarly to Lekatz et al. (2011). At lambing, birth weight, birth type, and sex were collected. Weaning weights were collected when the average lamb age was 85 ± 15 d.

Statistical analyses

Pregnancy, prolificacy, lambing rates, and weaning rates were analyzed using the GLIMMIX procedure of SAS (SAS Inst. Inc., Cary, NC). Birth BW, weaning BW, serum amino acid, and plasma progesterone concentrations were analyzed using the MXED procedure of SAS.

When analyzing weaning weights, the model included either birth type or weaning rate. The model included fixed effects of dietary treatment, d, and treatment × d. Significance was determined at $P \leq 0.05$. To partition treatment and d effects and treatment × d interactions, LS means and PDiffs were utilized ($P \leq 0.05$).

Results

No differences ($P \geq 0.39$; Table 2.4) were detected for pregnancy, prolificacy, or lambing rates among treatments. Weaning rate was greatest for IVARG treated ewes ($P = 0.05$) and least for RPARG ewes ($P = 0.05$). There were also no differences ($P \geq 0.21$) for lamb birth or adjusted weaning BW among treatments; however, when weaning weights were analyzed without number of lambs born in the model, there was a tendency ($P = 0.06$) for lambs born to IVARG ewes to have the largest weaning BW while lambs born to RPARG ewes had the smallest weaning BW.

There was a treatment and a day effect ($P < 0.0001$; $P = 0.0004$; respectively) detected in serum Arg concentrations, which increased from d 0 through d 14 with the exception of d 6 being less than d 4 ($P \leq 0.05$). The treatments exhibiting the highest ($P < 0.0001$) serum concentrations of Arg were IVALA, IVARG, and SBM with RPARG having the highest (202.13, 188.57, 206.92, 257.5 ± 24.9 μmol/L; respectively). Serum concentrations of citrulline (**Cit**) were highest ($P <0.0001$) for IVALA and IVARG treatments, with SBM being similar to IVARG (233.72, 214.70, and 204.97 ± 7.8 μmol/L; respectively); however, there was no d effect for Cit (P=0.96). Ornithine (**Orn**) serum concentrations were lower ($P <0.0001$) in CON and IVARG treated ewes (52.13 and 47.50 μmol/L, respectively), but exhibited no d effect (P=0.96). Two treatment × d interactions were observed for serum amino acid concentrations: hydroxylysine-2 (**Hyl2**; P=0.0007) and γ-amino-N-butyric acid (**GABA**; P= 0.0002). 0.04), with concentrations tending to decrease over time, although d 0 and 4 had no reported values.

Interestingly, GABA concentrations were also increased ($P < 0.0001$) for ewes receiving FM (9.88 ± 0.4 μmol/L) and also exhibited a d effect ($P = 0.04$) with concentrations tending to increase over the 14 d period. There were no other treatment × d interactions detected ($P \geq 0.77$) for serum amino acid concentrations for treatments (Table 2.5). The complete amino acid concentration profile is presented in the Appendix.

There was no treatment × day interaction ($P \geq 0.99$; Table 2.4) detected in plasma progesterone concentrations; however, there was a treatment ($P < 0.0001$) and a d effect ($P = 0.0004$; Figure 2.1), with progesterone concentrations increasing from d 0 through d 14 with the exception of d 8 being greater than d 10. The CON, IVALA, and RPARG ewes had similar levels of progesterone (2.79, 2.74, and 2.74; respectively); however, progesterone concentrations were decreased in ewes given FM, SBM, and the IVARG treatments. More interestingly, the RPARG treated ewes had higher ($P < 0.0001$) progesterone concentrations than the FM, SBM, and IVARG ewes.

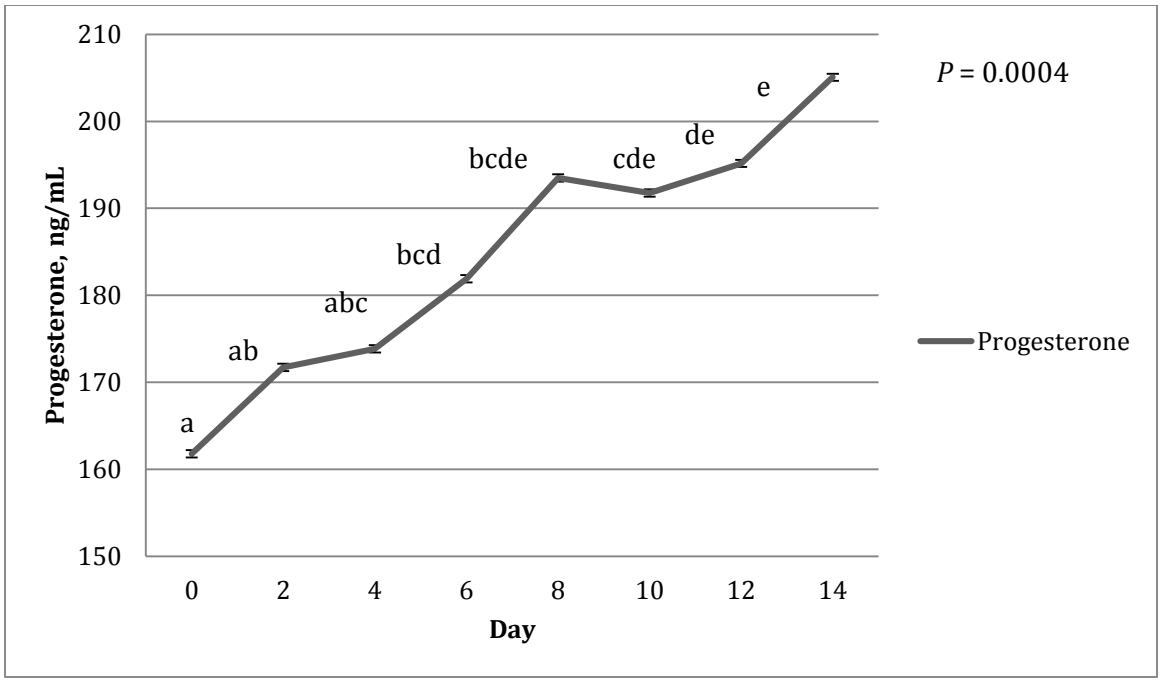

Figure 2.1. Effect of day on ewe plasma progesterone concentrations.

Table 2.4. Effects of daily injection of saline, L-alanine, L-arginine or oral supplementation of L-arginine 2 wk post-breeding on ewe performance

	Treatment[1]							
Item[3]	CON	IVALA	IVARG	RPARG	FM	SBM	SEM	*P*-value[2]
Pregnancy, %	88	90	88	86	88	83	11.6	0.99
Prolificacy	1.32	1.21	1.43	1.17	1.18	1.25	0.19	0.39
Lambing Rate	1.16	1.10	1.25	1.00	1.04	1.04	0.36	0.72
Weaning Rate	1.09^{bc}	0.95^{abc}	1.29^{c}	0.72^{a}	1.00^{abc}	0.86^{ab}	0.12	0.05
Progesterone, ng/mL[4]	2.79^{c}	2.74^{c}	2.38^{ab}	2.74^{c}	2.10^{a}	2.43^{b}	0.42	<0.0001

[1]CON = IV saline; IVALA = 30 mg • kg BW^{-1} • d^{-1} L-alanine; IVARG = 30 mg • kg BW^{-1} • d^{-1} L-arginine; RPARG = 454 g/d corn and 0.15 g • kg BW^{-1} • d^{-1} rumen-protected Arg; FM = 454 g/d 25:75 FM: corn; SBM = 454 g/d 37.5:62.5 SBM: corn.

[2]*P*-value for the overall F test of treatment (n = 25, 20, 23, 20, 24, and 23 for CON, IVALA, IVARG, RPARG, FM, and SBM respectively).

[3]Pregnancy = percent pregnant per ewe treated; Prolificacy= lambs per ewe lambed; Lambing rate = lambs per ewe treated; Weaning rate= lambs weaned per ewe lambing.

[4]Progesterone: n=12 per treatment.

[a,b,c] Means with different superscripts differ ($P \leq 0.05$) within each row; LS means were separated when *P*-value were ≤ 0.05 for treatment.

Table 2.5. Effects of daily injection of saline, L-alanine, L-arginine or oral supplementation of L-arginine 2 wk post-breeding on lamb performance

	Treatment[1]							
Item	CON	IVALA	IVARG	RPARG	FM	SBM	SEM	*P*-value[2]
Birth BW, kg	5.4	5.4	5.2	5.3	5.3	5.2	0.24	0.57
Weaning BW, kg[3]	24.6	19.3	26.1	15.4	20.8	18.0	2.69	0.06
AdjWeaning BW, kg[4]	26.7	22.8	26.9	19.3	24.5	21.1	3.04	0.21

[1]CON = IV saline; IVALA = 30 mg • kg BW^{-1} • d^{-1} L-alanine; IVARG = 30 mg • kg BW^{-1} • d^{-1} L-arginine; RPARG = 454 g/d corn and 0.15 g • kg BW^{-1} • d^{-1} rumen-protected Arg; FM = 454 g/d 25:75 FM: corn; SBM = 454 g/d 37.5:62.5 SBM: corn.

[2]*P*-value for the overall F test of treatment (n = 25, 20, 23, 20, 24, and 23 for CON, IVALA, IVARG, RPARG, FM, and SBM respectively).

[3]Weaning BW= litter weight or total ewe productivity.

[4]AdjWeaning BW= litter weight or total ewe productivity with number of lambs born in the model, (*P*-value for birth type ≤ 0.001).

[a,b,c] Means with different superscripts differ ($P \leq 0.05$) within each row; LS means were separated when *P*-value were ≤ 0.05 for treatment.

Table 2.6. Effects of daily injection of saline, L-alanine and L-arginine or oral supplementation of rumen-protected Arg, FM, SBM 2 wk post-breeding on ewe serum Ala, Arg, Cit, Orn, GABA, and Hyl2 concentrations

	Treatment[1]							*P*-value[2]	
Item, μmol/L[3]	CON	IVALA	IVARG	RPARG	FM	SBM	SEM	Trt	Day
Arg	174.4^{c}	202.1^{ab}	188.6^{bc}	257.5^{a}	161.5^{c}	206.9^{a}	24.9	<0.0001	0.0004
Cit	189.2^{ad}	233.7^{b}	172.6^{c}	211.0^{b}	183.1^{d}	205.0^{ac}	7.8	<0.0001	0.96
Orn	52.1^{ac}	58.8^{ad}	214.7^{bc}	5.0^{ae}	66.7^{bd}	66.4^{bd}	2.9	<0.0001	0.94
Ala	258.4^{a}	241.8^{acd}	192.3^{ad}	5.8^{e}	230.8^{bc}	229.3^{bd}	7.5	<0.0001	0.003
GABA	5.1^{ae}	3.9^{d}	47.5^{c}	0.77^{b}	9.9^{b}	4.7^{ad}	0.4	<0.0001	0.04
Hyl2	-	0.77^{b}	63.8^{bd}	0.64^{b}	1.19^{a}	0.74^{b}	0.05	<0.0001	0.04

[1]CON = IV saline; IVALA = 30 mg • kg BW^{-1} • d^{-1} L-alanine; IVARG = 30 mg • kg BW^{-1} • d^{-1} L-arginine; RPARG = 454 g/d corn and 0.15 g • kg BW^{-1} • d^{-1} rumen-protected Arg; FM = 454 g/d 25:75 FM: corn; SBM = 454 g/d 37.5:62.5 SBM: corn.

[2]*P*-value for the overall F test of treatment (n = 5, 5, 5, 5, 5, and 5 for CON, IVALA, IVARG, RPARG, FM, and SBM respectively). LS means were utilized to separate treatments differences when $P \leq 0.05$.

[3]GABA= gamma-amino-N-butyric acid and Hyl2= hydroxylysine 2.

[a,b,c] Means with different superscripts differ ($P \leq 0.05$) within each row; LS means were separated when *P*-value were ≤ 0.05 for treatment.

Discussion

The most important factor effecting success of most commercial sheep operations is reproductive efficiency (Lupton, 1998). New advancements in technology offer the possibility for significant improvements in efficiency of lamb production. With a volatile market continuing to potentially decrease profit margins, an increased emphasis, by producers and scientists alike, has been placed on maximizing lamb profitability.

Arginine is important for many biological functions, including synthesis of NO (Gouge et al., 1998; Manser et al., 2004). It has been hypothesized that treating ewes with Arg at, or slightly before, the time of maternal recognition of pregnancy may enhance the survival of the embryo during early embryogenesis (Luther et al., 2009). This is likely accomplished through the role Arg plays in polyamine and NO synthesis. Gestating sows supplemented with arginine achieved a 22% increase in live piglets born (11.4 vs. 9.4, $P < 0.03$, respectively; Mateo et al., 2007). Furthermore, pregnant rats supplemented with 1.3% Arginine-HCl throughout pregnancy

or between d 1 and 7 of gestation had increased embryonic survival and litter size by 30% (Zeng et al., 2008).

Pregnancy rates were not influenced through injectable or oral treatments in our study. Similarly, Luther et al., (2009) observed no differences in pregnancy rates for supplementation with injectable Arg from d 0 through d 14. In contrast, pregnancy rates were greater in ewes supplemented with injectable Arg from d 9 through 14 post-breeding (Saevre et al., 2011a). In the aforementioned studies (Luther et al., 2009; Saevre et al., 2011a), pregnancy rates ranged from 45-55%, whereas, pregnancy rates were approximately 88% for the current trial. The differences in pregnancy rates between these studies could be due to a difference in estrus synchronization techniques utilized. Ewes in the previous two studies were synchronized artificially with a controlled internal drug release inserts and an injection of PG600 (containing 400 IU of equine chorionic gonadotropin and 200 IU of human chorionic gonadotropin); whereas, the ewes in our study were naturally synchronized using ram exposure.

In the present study, only 1.5% of ewes displayed estrus prior to the start of the trial, indicating that most of the flock was in a period of anestrus before ram introduction. Gonzalez-Bulnes et al. (2005) gives evidence that progestagens, when compared with prostaglandin type products, might have negative effects on the functionality of ovulatory follicles. These follicles showed deficiencies in estradiol secretion during the preovulatory phase, which could prevent the oocyte from developing into a viable embryo (Gonzalez-Bulnes et al., 2005). Leoni et al. (2001) reported that ewes given superovulatory treatment with eCG/FSH had increased ovarian responses compared to FSH alone; however, the embryos showed a reduction in viability rates. In hamsters, embryos harvested from PMSG treated females and implanted into recipients showed a two-fold decrease in viability post-transfer when compared to non-stimulated

hamsters (McKiernan and Bavister, 1998). Although the aforementioned trials were all performed in embryo transfer trials, the results show that artificial usage of hormones can severely affect the viability of the embryos ovulating.

Prolificacy was not influenced through injectable or oral treatments in the present study. Similarly, Saevre et al. (2011a) reported no influence on prolificacy through injectable L-Arg. However, Luther et al., (2009) demonstrated differences in prolificacy with 1.6 lambs born per ewe treated with injectable Arg vs. 1.1 for the control ewes. We observed an average of 1.1 lambs born per ewe. Ovulation rates in out-of-season ewes have been shown to decrease by at least 15% when compared with in-season ewes (Glimp, 1971). Lunstra and Christenson (1981) demonstrated with administration of progesterone and PMSG, ovulation rates of out-of season ewes could be increased by 50%. Due to these varying results, more research is needed on the use of exogenous hormones in anestrous ewes and the effects of lambing rates for in-season and out-of-season ewes. It is possible that with hormonal induction to estrus, ewes might have increased ovulation rates. However, the oocytes can potentially be less viable. Ewes naturally induced to estrus, whether through light manipulation or ram introduction, have lower rates of ovulation, but the oocytes are healthier with increased viability. With limited research comparing naturally stimulated ewe's embryo quality to those artificially stimulated, it is difficult to make a definitive conclusion regarding this hypothesis. Arginine supplementation could be recovering these less viable embryos when in these compromised situations, but have no effect when embryos are not compromised in a "natural" model.

There were also no differences in adjusted weaning BW for our study and we are unaware of any prior Arg research that has reported weaning data. However, there was a tendency, when number of lambs born was not considered, for weaning weights IVARG treated

lambs to be increased. However, weaning rates for RPARG treated ewes were reduced when compared to the other treatments, while IVARG ewes had the greatest weaning rates. With the previous conclusion that Arg supplementation might possibly be recovering less viable embryos, is it possible that these lambs were born weak and not able to reach weaning? However, this is perplexing and quite speculative since the IVARG treated ewes tended to have the greatest weaning weights, and the RPARG lambs weights were still lowest. Additionally, the RPARG treatment could be providing too much Arg and up regulating NO synthesis, and therefore causing a toxicity to the lambs and compromising their future health and performance (Buhimschi et al., 1998). More research needs to be performed to address why these lambs underperformed.

Interestingly, Hyl2 concentrations were highest in ewes receiving FM, since FM is an excellent source of Hyl2. Although data for this amino acid is not commonly reported, it is a component in type III collagen and used for the structural components of vascularity. Therefore, increased levels of Hyl2 could be an indication of increased vascular growth occurring in FM supplemented ewes. Concentrations of GABA were also elevated in ewes receiving FM, which could have impacts on polyamine synthesis (McCann et al., 1979). McCann et al. (1979) reported that GABA, when added to rat hepatoma cells at the time of induction of cell proliferation, increased levels of ornithine decarboxylase (ODC) up to two- to three-fold when compared to control cells. The increase in ODC is also reflected by increases in intracellular putrescine levels, while spermidine and spermine were unchanged.

McCann et al. (1979) reported that GABAs stabilizing effect on ODC could be important in certain types of cells for the regulation of polyamine biosynthesis, which is important for placental development (Wu and Morris, 1998). Interestingly, serum levels of Arg

were only elevated in ewes receiving RPARG, IVARG, IVALA, and SBM, with only one of the supplemental Arg treatments succeeding in elevating serum levels. Saevre et al. (2011a) observed an increased level of serum Arg concentrations in ewes that received IVArg when compared to CON ewes'; however, Saevre et al. (2011b) reported that RPARG was provided at much higher concentrations of the diet and did increase serum Arg levels on d 11 and 12 of pregnancy when provided at 360 mg/kg BW compared to ewes on the CON diet, 90 mg/kg BW, and 180 mg/kg BW.

Citrulline concentrations were increased in IVALA and IVARG treated ewes, with SBM fed ewes being similar to IVARG levels. In contrast, Saevre et al. (2011a, b) reported no effects on Cit levels. A lack of increased levels of Cit by Saevre et al. (2011a, b) could be due to timing of serum samples being taken, since Cit is utilized for production of Arg and in circulation for a short period of time before being extracted from circulation by the kidney (Wu and Morris, 1998). It could also be due to an increased need for Arg synthesis, therefore an increase in production of Cit; however, this does not agree with the results from our current trial. It is possible that the increased Cit levels in IVARG, IVALA, and SBM treated ewes were causing the increase in serum Arg levels which were increased in these same treatments, since Cit is a precursor in the synthesis of Arg. However, further research would be needed to elucidate possible factors in the resulting concentration.

Ornithine concentrations were decreased in CON and IVARG ewes, which is interesting since increased Cit levels were observed in IVARG ewes and Orn is a precursor to Cit in the synthesis of Arg. However, this could be due to timing in blood sampling or possibly the ammonia pathway to synthesize Cit was utilized via carabmoyl-phosphate synthase I versus the Orn pathway (Wu and Morris, 1998). Treatments exhibiting increased Orn concentrations in

serum include: IVALA, RPARG, FM, and SBM. With the exception of IVALA, this increase in Orn concentration could be the result of a difference in the protein supply via feedstuffs.

In order for Arg supplementation to become more commercially applicable, an oral form of supplemental Arg should be developed. However, the results delivered thus far from oral supplementation have been in compromised maternal statuses and the results were equal to that of the control ewes. More research needs to be performed comparing challenged maternal statuses to see the true results of supplementation of Arg. The objective of this study was to determine the effects of injectable and oral Arg supplementation provided 2 wk post-breeding on reproductive performance of naturally stimulated fall lambing ewes. Our hypothesis was that supplemental Arg would increase pregnancy and lambing rates while decreasing postnatal lamb loss. In the current study, utilizing ewes synchronized for estrous through ram exposure for fall lambing, we reject the hypothesis that supplemental Arg will increase pregnancy rates and lambing rates; however, injectable Arg did increase weaning rates.

Implications

Although some previous research suggests that embryonic survival in sheep can be enhanced when ewes are supplemented with Arg, we did not detect any improvements in reproductive performance or lamb growth in ewes supplemented with injectable, non-rumen-protected, or rumen-protected forms of Arg; with the exception of IVARG improving weaning rates. Rumen-protected Arg should be investigated and examined to determine why weaning rates were decreased when supplemented to ewes and also why these sources did not yield the expected results. We also feel further research is warranted to determine rate of reproductive loss from different methods of non-seasonal estrus induction and the ability of supplemental Arg to recover these losses.

Literature Cited

AOAC Int. 1990. Official methods of analysis. 15th ed. AOAC Int., Arlington, VA.

AOAC Int. 2000. Official methods of analysis. 17th ed. AOAC Int., Arlington, VA.

AOAC Int. 2010. Official methods of analysis. 18th ed. AOAC Int., Arlington, VA.

Bolet, G. 1986. Timing and extent of embryonic mortality in pigs, sheep and goats: Genetic variability: in Embryonic Mortality in Farm Animals. J.M. Sreenan and M.G. Diskin, eds. Boston, MA: Dordrect. 12-43.

Brooks, M. A., J. H. Porter, and M. S. Kerley. 2011. Assessment of amino acid supplementation on rumen microbial efficiency and nitrogen metabolism using a continuous-culture system. Prof. Anim. Sci. 27:152-159.

Buhimschi, I. A., G. R. Saade, K. Chwalisz, and R. E. Garfield. 1998. The nitric oxide pathway in pre-eclampsia: pathophysiological implications. Human Reprod. Update. 4:25-42.

De Boo, H. A., P. L. van Zijl, D. E. Smith, W. Kulik, H. N. Lafeber, and J. E. Harding. 2005. Arginine and mixed amino acids increase protein accretion in the growth-restricted and normal ovine fetus by different mechanisms. Pediatr. Res. 58:270-277.

Dixon, A. B., M. Knights, J. L. Winkler, D. J. Marsh, J. L. Pate, M. E. Wilson, R. A. Dailey, G. Seidel, and E. K. Inskeep. 2007. Patterns of late embryonic and fetal mortality and association with several factors in sheep. J. Anim. Sci. 85:1274-1284.

Galbreath, C. W., E. J. Scholljegerdes, G. P. Lardy, K. G. Odde, M. E. Wilson, J. W. Schroeder, and K. A. Vonnahme. 2008. Effect of feeding flax or linseed meal on progesterone clearance rate in ovariectomized ewes. Domestic Anim. Endocrinol. 35:164-169.

Glimp, H. 1971. Effect of breed and mating season on reproductive performance of sheep. J. Anim. Sci. 32:1176-1182.

Gonzalez-Bulnes, A., A. Veiga-Lopez, P. Garcia, R. M. Garcia-Garcia, C. Ariznavarreta, M. A. Sanchez, J. A. F. Tresguerres, M. J. Cocero, and J. M. Flores. 2005. Effects of progestagens and prostaglandin analogues on ovarian function and embryo viability in sheep. Therio. 63:2523-2534.

Gouge, R. C., P. Marshburn, B. E. Gordon, W. Nunley, and Y. M. Huet-Hudson. 1998. Nitric oxide as a regulator of embryonic development. Biol. Reprod. 58:875-879.

Hulet, C. V., H. P. Voigtlander, A. L. Pope, and L. E. Casida. 1956. The nature of early-season infertility in sheep. J. Anim. Sci. 15:607-616.

Knights, M., Q. S. Baptiste, A. B. Dixon, J. L. Pate, D. J. Marsh, E. K. Inskeep, and P. E. Lewis. 2003. Effects of dosage of FSH, vehicle and time of treatment on ovulation rate and prolificacy in ewes during the anestrous season. Small Rum. Res. 50:1-9.

Lekatz, L. A., G. Wu, J. S. Caton, J. B. Taylor, L. P. Reynolds, D. A. Redmer, and K. A. Vonnahme. 2011. Maternal selenium supplementation and timing of nutrient restriction in pregnant sheep: Impacts on nutrient availability to the fetus. J. Anim. Sci. 89:59-76.

Leoni, G., L. Bogliolo, P. Pintus, S. Ledda, and S. Naitana. 2001. Sheep embryos derived from FSH/eCG treatment have a lower in vitro viability after vitrification than those derived from FSH treatment. Reprod. Nutr. Dev. 41:239-246.

Lunstra, D. D., and R. K. Christenson. 1981. Fertilization and embryonic survival in ewes synchronized with exogenous hormones during anestrous and estrous seasons. J. Anim. Sci. 53:458-466.

Lupton, C. J. 1998. ASAS CENTENNIAL PAPER: Impacts of animal science research on United States sheep production and predictions for the future. J. Anim. Sci. 86:3253-3274.

Luther, J. S., E. J. Windorski, J. S. Caton, G. Wu, J. D. Kirsch, K. A. Vonnahme, L. P. Reynolds, and C. S. Schauer. 2009. Effects of arginine supplementation on reproductive performance in Rambouillet ewes. NDSU Sheep Res. Rep. 50:11-13.

Manser, R. C., H. J. Leese, and F. D. Houghton. 2004. Effect of inhibiting nitric oxide production on mouse preimplantation embryo development on metabolism. Biol Reprod. 71:528-533.

Mateo, R. D., G. Wu, F. W. Bazer, J. C. Park, I. Shinzaro, and S. W. Kim. 2007. Dietary L-arginine supplementation enhances the reproductive performance of gilts. J. Nutr. 137:652-656.

McCann, P. P., J. M. Hornsperger, and N. Seiler. 1979. Regulatory interrelations between GABA and polyamines. II. Effect of GABA on ornithine decarboxylase and putrescine levels in cell culture. Neurochem. Res. 4:437-447.

McKiernan, S. H. and B. D. Bavister. 1998. Gonadotropin stimulation of donor females decreases post-implantation viability of cultured one-cell hamster embryos. Human Reprod. 13(3):724-729.

Moore, N. W., L. E. Rowson, and R. V. Short. 1960. Egg transfer in sheep. Factors affecting the survival and development of transferred eggs. J. Reprod. and Fertil. 1:322-349.

NRC. 2007. Nutrient requirements of small ruminants. Natl Acad Press, Washington, DC.

O'Mara, F. P., J. J. Murhy, and M. Rath. 1997. The amino acid composition of protein feedstuffs before and after ruminal incubation and after subsequent passage through the intestines of dairy cows. J. Anim. Sci. 75:1941-1949.

Quinlivan, T. D. 1966. Estimates of pre- and perinatal mortality in the New Zealand Romney Marsh ewe. J. Reprod. Fertil. 11:379-390.

Rhind, S. M., J. J. Robinson, C. Fraser, and I. McHattie. 1980. Relationships among uterine and placental factors in prolific ewes and their relevance to variations in fetal weight. Anim. Prod. 30:115-124.

Saevre, C., A. M. Meyer, M. L. Van Emon, D. A. Redmer, J. S. Caton, J. D. Kirsch, J. S. Luther, and C. S. Schauer. 2011a. Impacts of arginine on ovarian function and reproductive performance at the time of maternal recognition of pregnancy in ewes. NDSU Sheep Res. Rep. 52:13-16.

Saevre, C. B., J. S. Caton, J. S. Luther, A. M. Meyer, D. V. Dhuyvetter, R. E. Musser, J. D. Kirsch, M. Kapphahn, D. A. Redmer, and C. S. Schauer. 2011b. Effects of rumen-protected arginine supplementation on ewe serum-amino-acid concentration, circulating progesterone, and ovarian blood flow. Sheep and Goat Res J. 26:8-12.

Schrick F. N. and E. K. Inskeep. 1993. Determination of early pregnancy in ewes utilizing trans rectal ultrasonography. Theriogenology. 40:295-306.

Thureen, P. J., K A. Baron, P. V. Fennessey, and W. W. Hay Jr. 2002. Ovine placental and fetal arginine metabolism at normal and increased maternal plasma arginine concentrations. Pediatr. Res. 51:464-471.

Wu, G. and S. M. Morris. 1998. Arginine metabolism: nitric oxide and beyond. Biochem. J. 336:1-17.

Zeng, X., F. Wang, X. Fan, W. Yang, B. Zhou, P. Li, Y. Yin, G. Wu, and J. Wang. 2008. Dietary arginine supplementation during early pregnancy enhances embryonic survival in rats. J Nutr. 138:1304-1309.

APPENDIX. SERUM AMINO ACID CONCENTRATIONS

Table A.1. Effects of daily injection of saline, l-alanine and l-arginine or oral supplementation of rumen-protected Arg, FM, SBM 2 wk post-breeding on ewe serum amino acid concentrations

	Treatment[1]							*P*-value[2]	
Item, μmol/L[3]	CON	IVALA	IVARG	RPARG	FM	SBM	SEM	Trt	Day
β-Ala	5.05^{ab}	5.43^{b}	5.22^{b}	4.71^{a}	5.18^{b}	5.45^{b}	0.16	0.013	0.29
Thr	106.18^{ac}	109.14^{a}	101.88^{ac}	83.34^{b}	93.14^{bc}	102.02^{ac}	5.02	0.004	0.32
AADA	5.96^{a}	4.54^{bc}	5.36^{a}	4.72^{c}	4.53^{bc}	4.00^{b}	0.22	<0.0001	0.87
Pro	101.61^{ac}	106.14^{a}	106.60^{a}	87.06^{b}	86.68^{b}	98.72^{c}	2.71	<0.0001	0.19
AABA	13.46^{a}	12.56^{ab}	15.36^{c}	12.30^{b}	9.45^{d}	13.63^{a}	0.41	<0.0001	<0.0001
Cys	5.47^{a}	4.88^{abc}	5.17^{abc}	4.43^{bc}	4.44^{bc}	4.67^{abc}	0.29	0.09	0.97
Lys	123.96^{ac}	105.28^{bcd}	120.51^{ad}	110.80^{abd}	113.96^{abd}	108.71^{d}	4.96	0.08	0.008
Tyr	59.08^{a}	68.66^{b}	69.48^{b}	52.39^{a}	54.72^{a}	52.71^{a}	2.47	<0.0001	0.009
Met	19.67^{ac}	19.92^{ac}	20.82^{c}	16.62^{b}	18.77^{a}	18.59^{a}	0.64	0.0002	0.36
Val	214.97^{ac}	233.87^{bc}	243.86^{b}	200.89^{a}	202.45^{a}	226.22^{bc}	8.37	0.0011	0.88
Ile	82.54^{abc}	83.95^{cd}	91.20^{d}	76.15^{bc}	75.61^{b}	85.38^{da}	3.04	0.003	0.69
Leu	122.88^{ad}	129.65^{ac}	137.18^{c}	111.79^{bd}	111.44^{b}	121.41^{abd}	4.09	<0.0001	0.87
Phe	54.08^{a}	59.26^{b}	59.90^{b}	49.73^{a}	44.45^{c}	50.38^{a}	1.69	<0.0001	0.03
Trp	39.04^{ac}	44.63^{b}	46.27^{b}	37.63^{ac}	35.65^{a}	40.30^{c}	1.40	<0.0001	0.03
His	58.49^{a}	58.48^{ac}	57.43^{ac}	48.73^{b}	46.15^{b}	56.67^{ac}	1.37	<0.0001	0.62
Asn	63.91^{a}	60.13^{a}	58.35^{a}	47.93^{b}	47.04^{b}	57.54^{a}	2.42	<0.0001	0.02
3MH	26.93^{a}	21.84^{ac}	23.10^{a}	18.69^{bc}	14.06^{b}	21.49^{ac}	1.96	<0.0001	0.05
Tau	53.24^{a}	74.97^{b}	66.68^{b}	53.38^{a}	68.27^{b}	73.65^{b}	4.01	<0.0001	0.51
1MH	63.40^{a}	68.01^{acd}	72.07^{bd}	64.18^{a}	59.65^{a}	61.33^{a}	2.72^{a}	0.02	0.70
Ser	83.36^{a}	86.85^{a}	99.58^{c}	73.26^{d}	57.55^{b}	84.27^{a}	2.80	<0.0001	0.52
Gln	231.24^{a}	223.01^{ac}	249.12^{d}	199.46^{e}	223.01^{b}	213.28^{ce}	6.06	<0.0001	0.27
Car	26.94^{a}	21.55^{bc}	22.69^{b}	20.27^{b}	16.89^{c}	18.35^{c}	1.29	<0.0001	0.98
Gly	592.57^{a}	644.83^{c}	654.62^{a}	485.71^{bc}	415.97^{b}	571.90^{bc}	14.39	<0.0001	0.72
Ans	11.98^{a}	8.29^{c}	10.53^{c}	7.08^{d}	5.58^{b}	6.78^{a}	0.57	<0.0001	0.50
EA	4.43^{a}	5.01^{a}	4.87^{a}	4.37^{a}	3.39^{b}	4.41^{a}	0.235	<0.0001	0.11
Asp	14.70^{a}	13.35^{c}	14.32^{d}	14.11^{b}	12.09^{b}	11.67^{b}	0.54	0.0002	0.25
HyPro	15.26^{a}	18.92^{b}	19.23^{b}	13.60^{c}	15.28^{a}	16.00^{a}	0.58	<0.0001	0.90
Sar	3.83^{a}	4.19^{c}	4.56^{d}	3.25^{b}	2.99^{b}	3.27^{b}	0.11	<0.0001	0.12
Glu	88.69^{a}	78.52^{b}	85.19^{ad}	80.35^{bde}	84.70^{ace}	81.46^{bcd}	2.12	0.01	0.55

[1]CON = IV saline; IVALA = 30 mg • kg BW^{-1} • d^{-1} l-alanine; IVARG = 30 mg • kg BW^{-1} • d^{-1} l-arginine; RPARG = 454 g/d corn and 0.15 g • kg BW^{-1} • d^{-1} rumen-protected Arg; FM = 454 g/d 25:75 FM: corn; SBM = 454 g/d 37.5:62.5 SBM: corn.

[2]*P*-value for the overall F test of treatment (n = 5, 5, 5, 5, 5, and 5 for CON, IVALA, IVARG, RPARG, FM, and SBM respectively). LS means were utilized to separate treatments differences when $P \leq 0.05$.

[3]GABA= gamma-amino-N-butyric acid, AADA= alpha-amino-adipic acid, Hyl2= hydroxylysine 2, AABA= alpha-amino-N-butyric acid, 3MH= 3-methylhistidine, 1MH= 1-methylhistidine, Ans= anserine, EA= ethanolamine, HyPro= hydroxyproline.

[a,b,c] Means with different superscripts differ ($P \leq 0.05$) within each row; LS means were separated when *P*-value were ≤ 0.05 for treatment.