Amino Acids (2014) 46:375–384
DOI 10.1007/s00726-013-1626-6

ORIGINAL ARTICLE

Dietary supplementation with L-arginine between days 14 and 25 of gestation enhances embryonic development and survival in gilts

Xilong Li · Fuller W. Bazer · Gregory A. Johnson · Robert C. Burghardt · James W. Frank · Zhaolai Dai · Junjun Wang · Zhenlong Wu · Izuru Shinzato · Guoyao Wu

Received: 7 August 2013 / Accepted: 19 November 2013 / Published online: 13 December 2013

Abstract Embryonic loss is a major problem in mammals, but there are few effective ways to prevent it. Using a porcine model, we determined effects of dietary L-arginine supplementation between days 14 and 25 of gestation on embryonic growth and survival. Gilts were checked daily for estrus with boars in the morning and bred at onset of the second estrus and 12 h later (the time of breeding = day 0 of gestation). Between days 14 and 25 of gestation, 15 gilts/treatment were housed individually and fed twice daily 1 kg of a corn- and soybean meal-based diet supplemented with 0.0, 0.4, or 0.8 % L-arginine. All diets were made isonitrogenous by addition of L-alanine. On day 25 of gestation, gilts were hysterectomized to obtain conceptuses. Compared with controls, dietary supplementation with 0.4 or 0.8 % L-arginine increased ($P \leq 0.05$) arginine concentrations in maternal plasma, total volume of amniotic fluid; total amounts of arginine in allantoic and amniotic fluids; total amounts of fructose and most amino acids in amniotic fluid; placental growth; and the number of viable fetuses per litter by 2. The numbers of total fetuses, fetal weight, corpora lutea, volume of allantoic fluid, maternal circulating levels of progesterone and estrogen, or total amounts of hormones in allantoic fluid did not differ among the three treatment groups. Reproductive performance of gilts did not differ between the 0.4 and 0.8 % L-arginine groups. Thus, dietary supplementation with 0.4 or 0.8 % L-arginine between days 14 and 25 of gestation enhances embryonic/fetal survival in swine.

Keywords Amino acids · Conceptus · Gestation · Nutrition

Abbreviations

ALF	Allantoic fluid
AMF	Amniotic fluid
CL	Corpora lutea
NO	Nitric oxide
NRC	National Research Council

Introduction

Arginine serves as the physiological precursor for synthesis of proteins and other biological molecules, including ornithine, polyamines (putrescine, spermine and spermidine), proline, glutamine, creatine, agamatine, and nitric oxide (NO) (Wu and Morris 1998). Arginine and its metabolites have versatile functions in cardiovascular, neurological, immunological, and endocrine systems (Wu et al. 2009). Notably, results of recent studies led to the discovery that arginine can activate the mechanistic target of rapamycin cell signaling pathway (Yao et al. 2008; Kim et al. 2011), which plays crucial roles in protein synthesis, cell growth, and cytoskeletal remodeling (Bazer et al.

X. Li · F. W. Bazer · Z. Dai · J. Wang · G. Wu (✉)
Department of Animal Science, Texas A&M University, College Station 77843, TX, USA
e-mail: g-wu@tamu.edu

G. A. Johnson · R. C. Burghardt · J. W. Frank
Department of Veterinary Integrative Biosciences, Texas A&M University, College Station 77843, TX, USA

Z. Dai · J. Wang · Z. Wu · G. Wu
State Key Laboratory of Animal Nutrition, China Agricultural University, 100193 Beijing, China

I. Shinzato
Department of Animal Nutrition, Ajinomoto Co., Inc, 104-8315 Tokyo, Japan

2012). More importantly, NO and polyamines, two metabolites of arginine catabolism, may regulate conceptus survival and growth by promoting cell proliferation and migration, angiogensis, and dilation of blood vessels to increase blood flow (Wu et al. 2013c).

There is growing interest in an important role for L-arginine in regulating mammalian embryonic survival and growth (Ren et al. 2012; Satterfield et al. 2012, 2013; Wu et al. 2013a). For example, the number of live-born piglets is enhanced by 2 per litter in gilts receiving dietary supplementation with 0.83 % L-arginine between days 30 and 114 of gestation (Mateo et al. 2007). Similar results have been reported for multiparous sows receiving arginine supplementation between day 22 of gestation and parturition (Gao et al. 2012). Moreover, embryonic mortality in rats was reduced by 30 % in response to dietary supplementation with 1.2 % L-arginine for 7 days immediately after breeding (Zeng et al. 2008). These studies represent an important breakthrough for developing strategies to reduce embryonic loss, a major problem in reproduction of mammals, including pigs and humans. Early pregnancy (before day 25 of gestation) is the period when 75 % of embryonic losses occur (Pope 1994), and, therefore, this is a critical window to control embryonic mortality in pigs (Wu et al. 2010).

It was proposed that dietary supplementation with L-arginine during the first 25 days of pregnancy would ameliorate embryonic loss in pigs (Li et al. 2010). Unexpectedly, results from the previous study revealed that supplementing the diet of gilts with 0.8 % L-arginine between days 0 and 25 of gestation reduced reproductive performance as indicated by reductions in embryonic survival, number of corpora lutea (CL), and concentrations of progesterone in maternal plasma, as compared with the control group. The adverse effects of 0.8 % dietary L-arginine supplementation immediately after breeding suggest that excessive intake of dietary L-arginine interferes with CL formation or promote CL regression by increasing NO synthesis (Salvemini et al. 1993; Costa et al. 2008). This, in turn, reduces concentrations of progesterone in maternal plasma and the conceptus. Interestingly, there are reports that dietary supplementation with L-arginine for 2 weeks beginning on day 14 of gestation increases the number of viable fetuses in pigs (Bérard and Bee 2010). These results suggest that initiation of arginine supplementation after CL formation may capitalize on the benefits of arginine on conceptus survival and development without adverse effects on CL number and progesterone production. However, experimental results in support of this proposition are lacking. The present study tested the hypothesis that dietary supplementation with arginine between days 14 and 25 of gestation increases survival and development of conceptuses on day 25 of gestation.

Materials and methods

Chemicals

L-arginine and L-alanine were provided by Ajinomoto Co., Inc. (Tokyo, Japan). The RIA kits for progesterone (DSL-3400), estradiol (DLS-4400), estrone (DSL-8700), and estrone sulfate (DSL-5400) were obtained from Diagnostic Systems Laboratories (Webster, TX). Anticoagulant vacutainer tubes were procured from BD (Franklin Lakes, NJ). Amino acid standards for HPLC analysis were purchased from Sigma Chemicals (St. Louis, MO).

Animals and diets

The experimental design was similar to that described by Li et al. (2010), except that dietary supplementation with arginine was initiated on day 14 of gestation. Briefly, following breeding during the second period of estrus, 45 gilts (F1 crosses of Yorkshire X Landrace sows and Duroc X Hampshire boars) were assigned randomly to three treatment groups (0.0, 0.4, and 0.8 % L-arginine) and penned individually. Fifteen gilts were used for each treatment group. Between days 0 and 13 of gestation, gilts were fed twice daily (0700 and 1800 hours) 1 kg of a corn and soybean meal-based diet (2 kg diet/day). The basal diet met National Research Council (NRC)-recommended nutrient requirements for gestating gilts (NRC 1998). The basal diet was analyzed for amino acids as we described (Li et al. 2011) and contained 0.70 % arginine (Li et al. 2010). Starting on day 14 of gestation, gilts were fed twice daily (0700 and 1800 hours) 1 kg of a corn- and soybean meal-based diet supplemented with 0.0 (control), 0.4, or 0.8 % L-arginine (wt/wt) as described previously (Li et al. 2010). All diets were made isonitrogenous by addition of L-alanine.

Hysterectomy and tissue collection

On day 25 of gestation, 12 h after the last meal and 30 min after consumption of 16.4 g L-alanine (isonitrogenous control), 4 g L-arginine plus 8.2 g L-alanine plus 4.2 g cornstarch (0.4 % L-arginine group), and 8 g L-arginine plus 8.4 g cornstarch (0.8 % L-arginine group), gilts were prepared for anesthesia (~15 min) and then hysterectomized to obtain uteri and conceptuses after 10 mL samples of uterine venous and arterial blood were collected for analysis of metabolites and hormones (Li et al. 2010). Uterine weight, CL number, total number of fetuses, total number of viable fetuses, fetal weight and length, placental weight, and volumes of allantoic (ALF), and amniotic fluids (AMF) were measured and recorded (Wu et al. 1995, 1998a). ALF (10 mL) and all AMF from each fetus were collected for assays of metabolites and hormones (Li et al.

2010). No fetal blood samples could be obtained on day 25 of gestation due to the very small size of umbilical vessels. Portions of each placenta from the gilts were snap-frozen in liquid nitrogen. Endometrium was separated from myometrium using curved scissors and snap-frozen in liquid nitrogen (Wu et al. 1998b). All snap frozen samples were stored at −80 °C until analyzed.

Homogenization of placenta and endometrium

Frozen placenta (~200 mg) and endometrium (~100 mg) from eight gilts in each treatment group were homogenized with a glass homogenizer in 1 mL of ice-cold 1.5 mol/L $HClO_4$. The homogenate was transferred into 15 mL BD Falcon™ conical tubes. The homogenizer was rinsed twice each with 1 mL of 1.5 mol/L $HClO_4$. The combined homogenized solution was neutralized with 1.5 mL of 2 mol/L K_2CO_3. The solution was centrifuged at 10,000*g* for 2 min, and the supernatant fluid was used for HPLC analysis of amino acids.

Analysis of amino acids, fructose, electrolytes, and hormones

Amino acids in plasma from uterine artery plasma and fetal amniotic and allantoic fluids, as well as placental and endometrial extracts were analyzed by HPLC methods (Dai et al. 2012a, b). Values for cysteine included free cysteine and 1/2 cystine. Fructose was determined in duplicate as described by Roe (1934) with modifications. Briefly, 100 μL samples were deproteinized with 200 μL of 4.7 % trichloroacetic acid. The solution was centrifuged at 10,000*g* for 1 min, and the supernatant fluid was used for fructose assay. The reagents were added into a clear 96-well microplate in the order of 40 μL of sample or fructose standard, 40 μL of 1 mg/mL resorcinol, and 120 μL of 30 % HCl. A microplate was covered by a clear film followed by a gentle vortex (30 s) and incubation at 80 °C for 8 min. After the microplate cooled in running tap water, absorbance was measured at 490 nm. Fructose concentrations in samples were calculated on the basis of the fructose standard curve. Concentrations of electrolytes (sodium, potassium and calcium) in maternal uterine artery plasma and in allantoic fluid were determined using inductively coupled plasma-mass spectrometry (Department of Chemistry, Texas A&M University, College Station, TX, USA).

Progesterone, estradiol, estrone, and estrone sulfate in maternal uterine artery plasma and in fetal allantoic fluid were determined using RIA kits according to the instructions of the manufacturer. The minimum detection limit was 0.1 ng/mL, 4.7 pg/mL, 1.2 pg/mL, and 0.01 ng/mL for progesterone, estradiol, estrone, and estrone sulfate, respectively. The intra-assay coefficients of variation were 6.3, 4.6, 4.7, and 4.2 % for progesterone, estrone, and estrone sulfate assays, respectively. The inter-assay coefficients of variation were 9.2, 8.5, 5.4, and 10.7 % for progesterone, estradiol, estrone, and estrone sulfate assays, respectively.

Calculations and statistical analysis

The total amount of a substance in allantoic or amniotic fluid was calculated as concentration times the total volume of the fluid. Data, expressed as mean ± SEM, were analyzed using General Linear Model procedures of SPSS (Statistical Package for the Social Sciences; Version 15.0, Chicago, IL) for a randomized complete design. Gilt was considered as the experimental unit. Differences among treatment means were determined by the Student–Newman–Keuls multiple comparison test. Data on embryonic survival were analyzed using the Chi Square test of SPSS. Probability values ≤0.05 were considered statistically significant.

Results

Reproductive performance of gilts

One gilt from the control group and one gilt from the 0.8 % L-arginine group were not pregnant at the time of

Table 1 Reproductive performance of gilts fed diets supplemented with 0, 0.4 or 0.8 % L-arginine (Arg) between days 14 and 25 of gestation

Variable	Control	0.4 % Arg	0.8 % Arg	SEM	*P* value
Number of gilts (*n*)	14	15	14		
BW at breeding (kg)	119.9	113.3	110.9	4.8	0.762
BW on day 25 of gestation (kg)	121.3	114.4	112.1	4.8	0.745
BW gain (kg/25 days)	1.1	1.1	1.2	1.0	0.998
Uterine weight (kg)	2.46	2.66	2.48	0.07	0.389
Total fetuses (*n*)	11.3	12.8	12.4	0.4	0.228
Live fetuses (*n*)	10.5^{b}	12.7^{a}	12.2^{a}	0.4	0.050
CL (*n*)	13.9	14.4	13.6	0.3	0.586
Embryonic mortality (%)	24.7^{a}	11.2^{b}	10.1^{b}	1.9	0.001
Weight of viable fetuses (g)	5.58	6.27	5.83	0.18	0.252
Total placental weight (g)	93.0^{b}	124.6^{a}	112.5^{a}	4.3	0.004
Fetal length (cm)	1.84	1.82	1.81	0.02	0.872
Total ALF volume (L)	0.95	1.04	0.99	0.04	0.631
Total AMF volume (mL)	2.52^{b}	4.06^{a}	3.42^{a}	0.16	0.001

Values are means with pooled SEM; means in a row with superscripts without a common letter differ, $P < 0.05$

Table 2 Concentrations (μmol/L) of free amino acids in uterine artery plasma, allantoic fluid, and amniotic fluid of gilts fed diets supplemented with 0, 0.4, or 0.8 % L-arginine (Arg) between days 14 and 25 of gestation

Amino acid	Control	0.4 % Arg	0.8 % Arg	SEM	*P* value
Uterine artery					
Asp	18[a]	17[a]	11[b]	1.1	0.018
Glu	274[a]	246[a]	172[b]	15	0.008
Arg	161[b]	313[a]	336[a]	24	0.002
Ala	1114[a]	999[a]	437[b]	105	0.013
Orn	79[b]	123[a]	119[a]	7.5	0.026
Allantoic fluid					
Arg	104[b]	154[a]	191[a]	12	0.006
Ala	196	217	187	12	0.564
Amniotic fluid					
Asp	25[a]	24[a]	16[b]	1.6	0.026
Glu	266[a]	218[a]	139[b]	15	0.001
Arg	118	114	120	5.5	0.910
Ala	361	381	336	13	0.404

Values are means and pooled SEM, $n = 10$; means in a row with superscripts without a common letter differ, $P < 0.05$

Table 3 Concentrations (μmol/L) of free amino acids in uterine artery plasma of gilts fed diets supplemented with 0, 0.4 or 0.8 % L-arginine between days 14 and 25 of gestation

Amino acids	Control	0.4 % L-Arginine	0.8 % L-Arginine	SEM	*P* value
Asn	82	70	71	2.9	0.215
Ser	130	122	112	4.9	0.366
Gln	544[a]	512[a,b]	456[b]	24	0.047
His	90	95	93	3.5	0.878
Gly	734	643	676	54	0.798
Thr	190	169	167	8.1	0.457
Cit	71	76	62	3.6	0.291
Beta-Ala	25	21	16	1.8	0.114
Tau	75	79	70	5.5	0.082
Tyr	107	95	93	3.0	0.075
Trp	62	55	56	2.6	0.531
Met	44	40	39	1.7	0.539
Val	311	283	278	11	0.424
Phe	80	71	73	2.5	0.308
Ile	124	111	113	4.6	0.499
Leu	200	186	179	6.5	0.427
Lys	217	202	229	9.9	0.558
Pro	252	234	247	8.4	0.663
Cys	205	236	240	16	0.623
OH-Pro	19	19	18	1.0	0.940

Values are means and pooled SEM, $n = 10$

[a,b] $P < 0.05$

hysterectomy (Table 1). There were no differences among the three groups of gilts in maternal body-weight (BW) gain, total uterine weight, total number of fetuses, number of CL, total fetal weight, fetal length, or volume of ALF (Table 1). However, compared with the control group, dietary supplementation with 0.4 or 0.8 % L-arginine increased placental weight by 21 and 34 % ($P < 0.01$), respectively, and the number of live fetuses per litter by 2 ($P = 0.05$), while reducing ($P < 0.01$) embryonic mortality by 14 and 15 %, respectively (Table 1). The total volume of AMF was greater ($P < 0.01$) for conceptuses from gilts supplemented with either 0.4 or 0.8 % L-arginine (Table 1). Reproductive performance of gilts did not differ between the 0.4 and 0.8 % L-arginine groups.

Concentrations of amino acids in maternal plasma

Gilts in the 0.4 and 0.8 % L-arginine groups had higher ($P < 0.05$) concentrations of arginine and ornithine in maternal plasma, compared with the control group (Table 2). Concentrations of aspartate, glutamate, glutamine and alanine were lower ($P < 0.05$) in plasma from

Table 4 Concentrations (μmol/L) of free amino acids in allantoic fluid of gilts fed diets supplemented with 0, 0.4, or 0.8 % L-arginine (Arg) between days 14 and 25 of gestation

Amino acids	Control	0.4 % Arg	0.8 % Arg	SEM	*P* value
Asp	8	8	7	0.5	0.735
Glu	64	62	47	4.3	0.209
Asn	89	84	78	4.7	0.647
Ser	526	544	461	27	0.451
Gln	699	677	597	43	0.613
His	94	89	92	6.1	0.935
Gly	460	401	414	26	0.645
Thr	206	196	204	13	0.945
Cit	10.3	10.1	9.5	0.8	0.916
β-Ala	33	28	28	1.4	0.319
Tau	432	396	392	29	0.835
Tyr	79	74	68	3.1	0.369
Trp	12	14	13	0.8	0.842
Met	15	15	16	0.9	0.934
Val	88	86	87	5.6	0.991
Phe	34	34	37	2.8	0.911
Ile	24	23	24	1.6	0.977
Leu	50	48	47	3.7	0.966
Orn	141	136	145	7.4	0.904
Lys	299	291	321	19	0.801
Pro	248	271	234	13	0.546
Cys	42	39	44	2.7	0.802
OH-Pro	63	70	59	2.6	0.242

Values are means and pooled SEM, $n = 10$

Table 5 Total amounts (μmol) of free amino acids in allantoic fluid of gilts fed diets supplemented with 0, 0.4, or 0.8 % L-arginine (Arg) between days 14 and 25 of gestation

Amino acids	Control	0.4 % Arg	0.8 % Arg	SEM	*P* value
Asp	7	8	7	0.5	0.803
Glu	59	61	44	5.2	0.394
Asn	78	82	75	6.2	0.898
Ser	467	524	437	36	0.629
Gln	614	661	585	54	0.854
His	79	85	87	6.5	0.868
Gly	400	390	394	29	0.991
Thr	171	192	195	15	0.804
Cit	8.8	9.0	9.2	0.8	0.980
Arg	106[b]	168[a]	195[a]	14	0.008
β-Ala	28	27	27	1.5	0.929
Tau	351	361	372	24	0.940
Ala	195	209	189	12	0.805
Tyr	69	71	66	4.7	0.891
Trp	11	13	12	1.0	0.567
Met	13	14	15	1.1	0.815
Val	74	84	84	6.6	0.810
Phe	29	33	36	3.0	0.653
Ile	20	23	23	2.0	0.801
Leu	42	46	45	3.8	0.876
Orn	118	131	135	7.8	0.659
Lys	249	284	302	22	0.600
Pro	221	262	225	18	0.619
Cys	36	40	41	3.3	0.867
OH-Pro	62	62	60	4.6	0.974

Values are means and pooled SEM, $n = 10$; means in a row with superscripts without a common letter differ, $P < 0.05$

Table 6 Concentrations (μmol/L) of free amino acids in amniotic fluid of gilts fed diets supplemented with 0, 0.4, or 0.8 % L-arginine (Arg) between days 14 and 25 of gestation

Amino acids	Control	0.4 % Arg	0.8 % Arg	SEM	*P* value
Asn	104	98	101	3.8	0.789
Ser	513	537	499	21	0.781
Gln	1,235	1,121	977	48	0.093
His	68	74	78	3.7	0.481
Gly	392	446	430	17	0.442
Thr	214	231	230	11	0.778
Cit	22	21	21	0.9	0.873
β-Ala	27	26	26	1.2	0.882
Tau	204	249	221	12	0.255
Tyr	121	117	116	3.5	0.864
Trp	18	17	18	0.7	0.587
Met	57	55	55	1.5	0.859
Val	227	220	219	9.0	0.938
Phe	86	80	84	4.5	0.869
Ile	63	60	62	2.4	0.882
Leu	157	151	160	5.5	0.824
Orn	78	87	74	3.6	0.283
Lys	174	168	168	7.7	0.942
Pro	199	214	196	9.2	0.728
Cys	26	26	27	1.3	0.945
OH-Pro	39	44	38	1.3	0.144

Values are means and pooled SEM, $n = 10$

gilts supplemented with 0.8 % L-arginine compared with control gilts, but the values did not differ between the 0.4 % arginine and control groups (Table 2). Concentrations of other amino acids in maternal plasma did not differ among the three treatment groups of gilts (Table 3).

Concentrations and total amounts of amino acids in allantoic and amniotic fluids

Concentrations of arginine in ALF were greater ($P < 0.05$) in conceptuses from gilts supplemented with 0.4 and 0.8 % arginine, compared with control gilts (Table 2). Concentrations of other amino acids in ALF did not differ among the three groups of gilts (Table 4). Total amounts of arginine in ALF increased ($P < 0.01$) in response to dietary supplementation with 0.4 or 0.8 % L-arginine, but total amounts of other amino acids (including alanine, glutamine, proline, and lysine) in ALF were not affected by arginine supplementation (Table 5).

Concentrations of both aspartate ($P < 0.05$) and glutamate ($P < 0.01$) were greater in AMF of gilts supplemented with 0.8 % arginine, as compared with the control and 0.4 % arginine groups (Table 2). No difference was detected for concentrations of other amino acids (including arginine, alanine, and lysine) in AMF among the three treatment groups (Tables 2, 6). Compared with the control group, total amounts of arginine and most of other amino acids in AMF were greater ($P < 0.05$) for conceptuses from gilts supplemented with 0.4 and 0.8 % arginine (Table 7).

Concentrations of amino acids in placentae and endometria

Concentrations of arginine and proline in the placenta were greater ($P < 0.05$), but concentrations of alanine were lower ($P < 0.01$), in gilts supplemented with 0.8 % L-arginine compared with control gilts and the gilts supplemented with 0.4 % arginine (Table 8). Similar results were obtained for concentrations of proline and alanine in endometrial samples (Table 9). Concentrations of other amino acids in placentae and endometria did not differ among the treatment groups of gilts (Tables 8, 9).

Table 7 Total amount (μmol) of free amino acids in amniotic fluid of gilts fed diets supplemented with 0, 0.4, or 0.8 % L-arginine (Arg) between days 14 and 25 of gestation

Amino acids	Control	0.4 % Arg	0.8 % Arg	SEM	*P* value
Asp	76	84	53	6.0	0.061
Glu	674[a]	815[a]	471[b]	50	0.007
Asn	263	346	333	20	0.192
Ser	1280[b]	2090[a]	1642[ab]	124	0.033
Gln	3294[b]	4136[a]	3227[b]	187	0.032
His	163[b]	285[a]	274[a]	20	0.026
Gly	1065[b]	1849[a]	1660[a]	136	0.050
Thr	497[b]	889[a]	846[a]	66	0.021
Cit	58	72	68	6.0	0.152
Arg	303[b]	492[a]	425[a]	28	0.010
β-Ala	68	96	87	6.9	0.245
Tau	538[b]	967[a]	715[ab]	68	0.022
Ala	1043[b]	1436[a]	1059[b]	72	0.026
Tyr	294[b]	431[a]	363[ab]	21	0.015
Trp	46[b]	60[a]	66[a]	3.1	0.016
Met	159	186	193	14	0.242
Val	533[b]	815[a]	833[a]	49	0.012
Phe	221	295	284	19	0.250
Ile	147[b]	214[a]	209[a]	12	0.035
Leu	401[b]	564[a]	521[a]	31	0.012
Orn	183[b]	342[a]	216[a]	22	0.001
Lys	445[b]	654[a]	514[ab]	38	0.049
Pro	532[c]	857[a]	664[b]	65	0.001
Cys	75[b]	126[a]	97[ab]	7.4	0.010
OH-Pro	98[b]	176[a]	136[ab]	12	0.020

Values are means and pooled SEM, $n = 10$; means in a row with superscripts without a common letter differ, $P < 0.05$

Table 8 Concentrations (nmol/mg tissue) of free amino acids in placentae of gilts fed diets supplemented with 0, 0.4, or 0.8 % L-arginine (Arg) between days 14 and 25 of gestation

Amino acids	Control	0.4 % Arg	0.8 % Arg	SEM	*P* value
Asp	0.46	0.31	0.36	0.030	0.126
Glu	0.82	0.70	0.69	0.120	0.596
Asn	0.20	0.14	0.17	0.011	0.094
Ser	0.57	0.48	0.46	0.048	0.432
Gln	2.44	1.90	1.85	0.124	0.596
His	0.24	0.20	0.15	0.024	0.328
Gly	0.96	0.86	0.85	0.053	0.649
Thr	0.43	0.36	0.38	0.032	0.671
Cit	0.025	0.019	0.021	0.008	0.104
Arg	0.29[b]	0.30[b]	0.36[a]	0.020	0.034
Tau	1.01	0.90	0.91	0.063	0.755
Ala	0.59[a]	0.54[a]	0.41[b]	0.026	0.001
Tyr	0.17	0.20	0.16	0.016	0.867
Trp	0.05	0.06	0.05	0.005	0.633
Met	0.08	0.08	0.07	0.006	0.438
Val	0.33	0.31	0.32	0.019	0.515
Phe	0.15	0.14	0.13	0.013	0.770
Ile	0.12	0.12	0.10	0.008	0.331
Leu	0.24	0.23	0.19	0.037	0.902
Orn	0.13	0.11	0.11	0.006	0.193
Lys	0.38	0.41	0.37	0.034	0.338
Cys	0.24	0.25	0.27	0.015	0.372
Pro	0.27[b]	0.28[b]	0.32[a]	0.011	0.010

Values are means with pooled SEM, $n = 8$; means in a row with superscripts without a common letter differ, $P < 0.05$

Hormones and electrolytes in uterine artery plasma and ALF

Progesterone was more abundant ($P < 0.01$) than estradiol, estrone, and estrone sulfate in maternal plasma (Table 10). In contrast, concentrations of estrone sulfate in ALF were approximately 50-fold greater ($P < 0.01$) than concentrations of progesterone. Concentrations and total amounts of progesterone, estradiol, estrone, and estrone sulfate (Table 10) as well as concentrations of sodium, potassium and calcium (Table 11) in maternal uterine artery plasma did not differ among the three treatment groups of gilts. Dietary supplementation with 0.4 or 0.8 % L-arginine increased the concentration and total amount of sodium in allantoic fluid, compared with the control group (Table 11).

Fructose in ALF and AMF

Concentrations of fructose in ALF were similar to those in AMF (Table 12). Concentrations of fructose in ALF and AMF did not differ among the treatment groups of gilts. Likewise, dietary supplementation with arginine did not affect total amounts of fructose in ALF (Table 12). However, total amounts of fructose were greater ($P < 0.01$) in AMF of gilts receiving dietary supplementation with 0.4 or 0.8 % L-arginine, compared with the control group. Neither concentrations nor total amounts of fructose in ALF and AMF differed between the 0.4 and 0.8 % arginine groups (Table 12).

Discussion

Embryonic loss is a major problem in mammals, including humans and swine (Ren et al. 2013; Wu et al. 2013a). Unfortunately, there are few effective ways to reduce such a high loss of embryos during gestation (Bazer et al. 2012). As noted previously, L-arginine is a functional amino acid in animal nutrition (Wu 2013a) and a ratio of arginine:lysine in the swine diet should not be greater than 3.0 (Go et al. 2012; Wu 2013b). Interestingly, dietary supplementation with 1.0 % arginine-HCl between days 30 and

Table 9 Concentrations (nmol/mg tissue) of amino acids in endometria of gilts fed diets supplemented with 0, 0.4, or 0.8 % L-arginine between days 14 and 25 of gestation

Amino acids	Control	0.4 % Arg	0.8 % Arg	SEM	*P* value
Asp	0.74	0.59	0.64	0.117	0.720
Glu	2.05	1.80	1.83	0.075	0.370
Asn	0.064	0.069	0.063	0.004	0.588
Ser	0.29	0.32	0.23	0.018	0.076
Gln	0.86	0.80	0.87	0.048	0.804
His	0.19	0.19	0.14	0.013	0.302
Gly	1.88	1.78	1.76	0.086	0.829
Thr	0.24	0.23	0.23	0.008	0.657
Cit	0.068	0.072	0.064	0.004	0.572
Arg	0.27	0.29	0.30	0.024	0.129
Tau	2.93	2.79	2.53	0.159	0.612
Ala	1.35[a]	1.59[a]	0.74[b]	0.128	0.012
Tyr	0.16	0.14	0.15	0.008	0.538
Trp	0.026	0.024	0.024	0.002	0.187
Met	0.067	0.064	0.085	0.009	0.109
Val	0.31	0.31	0.29	0.010	0.617
Phe	0.10	0.092	0.084	0.005	0.208
Ile	0.19	0.16	0.12	0.011	0.067
Leu	0.33	0.31	0.24	0.018	0.147
Orn	0.082	0.10	0.084	0.008	0.373
Lys	0.25	0.22	0.23	0.009	0.728
Cys	0.24	0.26	0.25	0.012	0.511
Pro	0.28[b]	0.29[b]	0.33[a]	0.009	0.002

Values are means with pooled SEM, $n = 8$; means in a row with superscripts without a common letter differ, $P < 0.05$

Table 10 Concentrations of hormones in uterine artery plasma and total amounts of the hormones in allantoic fluid in gilts fed diets supplemented with 0, 0.4, or 0.8 % L-arginine (Arg) between days 14 and 25 of gestation

Hormones	Control	0.4 % Arg	0.8 % Arg	SEM	*P* value
Uterine artery plasma					
Progesterone (ng/ml)	16.3[a]	17.9[a]	17.9[a]	1.14	0.817
Estradiol (ng/ml)	0.102[c]	0.109[c]	0.087[c]	0.008	0.550
Estrone (ng/ml)	0.127[c]	0.113[c]	0.089[c]	0.008	0.142
Estrone sulfate (ng/ml)	9.22[b]	9.07[b]	6.19[b]	0.74	0.174
Allantoic fluid					
Progesterone (μg)	1.13[b]	1.22[b]	1.14[b]	0.10	0.923
Estradiol (μg)	0.671[c]	0.631[c]	0.608[c]	42.7	0.842
Estrone (μg)	0.615[c]	0.590[c]	0.582[c]	58.2	0.974
Estrone sulfate (μg)	60.6[a]	58.7[a]	63.7[a]	4.57	0.909

Values are means with pooled SEM, $n = 14$ gilts. For uterine artery plasma or ALF, means in a column with superscripts without a common letter differ, $P < 0.05$

Table 11 Concentrations of ions in maternal uterine artery plasma and fetal allantoic fluid of gilts fed diets supplemented with 0, 0.4 or 0.8 % L-arginine (Arg) between days 14 and 25 of gestation

Variable	Control	0.4 % Arg	0.8 % Arg	SEM	*P* value
Concentrations in maternal plasma					
Na^+ (mmol/L)	140	137	138	3.24	0.798
K^+ (mmol/L)	4.32	4.16	4.28	0.15	0.737
Ca^{2+} (mmol/L)	8.07	8.14	8.02	0.22	0.928
Concentrations in ALF					
Na^+ (mmol/L)	21.6[b]	25.9[a]	26.4[a]	0.83	0.001
K^+ (mmol/L)	6.95	6.73	6.78	0.18	0.667
Ca^{2+} (mmol/L)	1.28	1.23	1.20	0.06	0.640
Total amounts in ALF					
Na^+ (mmol)	20.4[b]	26.8[a]	26.2[a]	0.94	0.001
K^+ (mmol)	6.62	7.01	6.71	0.20	0.366
Ca^{2+} (mmol)	1.22	1.29	1.19	0.07	0.590

Values are means with pooled SEM, $n = 10$; means in a row with superscripts without a common letter differ, $P < 0.05$

114 of gestation increased the number of live-born piglets per litter by 2 (Mateo et al. 2007). Although there are two critical windows for fetal death after day 30 of gestation, more than 75 % of prenatal loss occurs during the first 25 days of gestation (Pope 1994; Bazer and Thatcher 1977). This implicates that dietary L-arginine supplementation may have positive effects on fertility when it is initiated during early pregnancy because it is the most critical window of opportunity to control embryonic mortality in pigs. However, results of our previous work (Li et al. 2010) indicated that the number of live fetuses, CL number, and concentrations of progesterone in maternal plasma were decreased by supplementing 0.8 % L-arginine to the diet of gilts between days 0 and 25 of gestation. In contrast, results of this study with arginine supplementation beginning on day 14 of gestation indicated that dietary supplementation with 0.4 or 0.8 % L-arginine did not affect number of CL, but did increase the number of live fetuses and decrease embryonic mortality on day 25 of gestation as compared with control gilts (Table 1). The major difference in experimental design between the present and previous studies is the day when L-arginine supplementation was initiated (day 14 in the present study versus day 0 in the previous study). A decrease in CL number in response to L-arginine supplementation immediately after breeding may have resulted from the interference with growth and ovulation of ovarian follicles and formation of CL (Smith et al. 1994). Maternal recognition of pregnancy starts on day 11 of pregnancy in swine when blastocysts begin their dramatic morphological changes (Bazer and Thatcher 1977). Supplementing the diet with 0.8 % L-arginine before

day 14 of gestation may impede this process, resulting in decreased embryonic survival. However, when dietary L-arginine supplementation starts on day 14 of gestation, CL formation and maternal recognition of pregnancy are not affected by L-arginine supplementation.

Nutrition can affect fertility in mammals (Schoknecht et al. 1993, 1994). Interestingly, Ramaeker et al. (2006) reported that dietary supplementation with 1 % arginine from days 14 to 28 increased the number of live-born piglets at birth by approximately 1 per sow. The results from the present study indicate that enhanced embryonic survival before day 25 of gestation is a major factor contributing to increased litter size at term. It is known that some fetal loss can occur between day 25 of gestation and parturition in gilts and sows with large litters early in gestation (Wu et al. 2006). Daily supplementation with arginine beyond day 25 of gestation until term will help prevent fetal loss and increase litter size to a greater extent (approximately one more live-born piglet per litter), when compared with dietary arginine supplementation between days 14 and 25 of gestation. Enhanced growth (Table 1) and vascularization of the placentae (Li et al. 2010) likely promote embryonic survival in arginine-supplemented gilts and positively impact fetal survival and growth in subsequent stages of gestation. This further supports the notion that placental angiogenesis, size, and function are crucial for successful pregnancy outcome in mammals (Liu et al. 2012; Reynolds et al. 2005; Vonnahme and Ford 2004). Note that total fetal weight on day 25 of gestation was not affected by dietary supplementation with arginine alone, likely because the embryos receive nutrients primarily from uterine secretions during early pregnancy.

Another important finding from this study is that CL number was not affected by dietary supplementation with arginine between days 14 and 25 of gestation. Similarly, no difference was detected in concentrations of progesterone in maternal plasma among the three groups of gilts. The CL are the only source of progesterone in pigs and it is essential for normal pregnancy (Bazer et al. 2012). Estrogen, another important hormone in pregnancy, was not affected by L-arginine supplementation (Table 10). Thus, an increase in embryonic survival in arginine-supplemented gilts is likely mediated by factors other than progesterone and estrogen. These factors may include a regulatory role of arginine in the mammalian (mechanistic) target of rapamycin signaling in the conceptus (Kim et al. 2011; Kong et al. 2012), as reported for leucine and glutamine in skeletal muscle and the small intestine (Rhoads and Wu 2009; Suryawan and Davis et al. 2011; Xi et al. 2011). However, based on results of the previous study (Li et al. 2010), it is clear that adequate amounts of progesterone are necessary for arginine to enhance embryonic survival during early gestation.

Table 12 Concentrations and total amounts of fructose in fetal fluids of gilts fed diets supplemented with 0, 0.4, or 0.8 % L-arginine (Arg) between days 14 and 25 of gestation

Variable	Control	0.4 % Arg	0.8 % Arg	SEM	*P* value
Fructose (mg/ml)					
ALF	1.13	1.13	1.09	0.04	0.884
AMF	0.76	0.88	0.82	0.03	0.277
Total fructose (mg)					
ALF	1,045	1,178	1,099	63	0.682
AMF	1.93[b]	3.38[a]	2.84[a]	0.18	0.001

Values are means with pooled SEM, $n = 14$ gilts; means in a row with superscripts without a common letter differ, $P < 0.05$

The total volume of AMF increased by 36–61 % in gilts receiving diets supplemented with 0.4 or 0.8 % L-arginine, compared with the control group (Table 1). Similarly, dietary supplementation with 0.4 and 0.8 % L-arginine increased the total amount of sodium in fetal allantoic fluid by 31 %. The mechanisms responsible for enhanced transport of water, arginine, most other amino acids, sugars, and ions across placentae, as well as amniotic and allantoic membranes are unclear. Results of this study suggest an important role for arginine in regulating these physiological processes in the conceptus, which is in keeping with the versatile functions of arginine in nutrient metabolism (Geng et al. 2011; McKnight et al. 2010; Wu et al. 2013b). Total amounts of fructose in AMF also increased in the 0.4 or 0.8 % L-arginine groups as compared with the control group (Table 12). It is possible that arginine stimulates the conversion of glucose to fructose in placentae and subsequent transport of fructose across the fetal side of the placenta into the amniotic fluid. While the underlying mechanisms remain to be elucidated, our observation is novel and important, as fructose is now known to be a substrate for the synthesis of glycoproteins in porcine placentae (Li 2011) and to stimulate cell signaling in porcine trophectoderm cells (Bazer et al. 2012). Fructose does not move from the fetal system to maternal circulation (Bazer 1989), but plays an important role in cell signaling pathways (Kim et al. 2012). Thus, an increase in availability of this sugar in conceptuses of arginine-supplemented gilts warrants further investigation.

Consistent with the previous study (Li et al. 2010), concentrations of aspartate, glutamate, glutamine and alanine were lower, but concentrations of arginine and ornithine were higher in plasma of gilts supplemented with 0.8 % L-arginine compared with control gilts (Table 2). Moreover, gilts in the 0.4 % L-arginine group had higher circulating levels of arginine than control gilts (Table 2). It is possible that rates of synthesis of glutamine, aspartate

and glutamate were greater, or rates of degradation of these amino acids were lower in the whole body of control gilts (receiving isonitrogenous amounts of alanine) than in gilts supplemented with 0.8 % arginine. The findings that embryonic survival was greater in gilts supplemented with 0.4 % arginine than control gilts even though concentrations of alanine, glutamine, aspartate or glutamate in maternal plasma did not differ between these two groups indicate that the circulating levels of alanine, aspartate, glutamate and glutamine in blood did not affect litter size in gilts (Li et al. 2010). Thus, dietary supplementation with 0.4 % L-arginine is sufficient to enhance reproductive performance in gilts as is 0.8 % L-arginine. This new knowledge is very important for developing a cost-effective strategy to enhance swine production worldwide.

In summary, supplementing the diet of gilts with 0.4 or 0.8 % L-arginine between days 14 and 25 of gestation increased concentrations of arginine in maternal plasma, total amounts of arginine in ALF and AMF, and the number of live fetuses per litter by 2 on day 25 as compared with control gilts. Arginine supplementation also increased the volume of AMF, total amounts of fructose and most amino acids in AMF and the total amount of sodium in ALF possibly due to enhanced transport of ions, water, sugar, and amino acids across placentae and into the fetal fluids. These findings will aid in developing cost-effective strategies to enhance litter size in swine and also have important implications for improving embryonic survival in other mammals.

Acknowledgments This study was supported by National Research Initiative Competitive Grants from the Animal Reproduction Program (2008-35203-19120) and Animal Growth & Nutrient Utilization Program (2008-35206-18764) of the USDA National Institute of Food and Agriculture, Texas AgriLife Research (No. 8200), National Natural Science Foundation of China (31272217 and 31272450), Chinese Universities Scientific Funds (2012RC024), and the Thousand-People-Talent program at China Agricultural University.

Conflict of interest The authors declare no conflicts of interest.

References

Bazer FW (1989) Allantoic fluid: regulation of volume and composition. In: Brace RA, Ross MG, Robillard JE (eds) Reproduction and perinatal medicine. Fetal and neonatal body fluids. Ithaca: Perinatology, vol. 11, p 135–155

Bazer FW, Thatcher WW (1977) Theory of maternal recognition of pregnancy in swine based on estrogen controlled endocrine versus exocrine secretion of prostaglandin F2alpha by the uterine endometrium. Prostaglandins 14:397–401

Bazer FW, Wu G, Spencer TE et al (2010) Novel pathways for implantation and establishment and maintenance of pregnancy in mammals. Mol Hum Reprod 16:135–152

Bazer FW, Song GH, Kim JY et al (2012) Mechanistic mammalian target of rapamycin (MTOR) cell signaling: effects of select nutrients and secreted phosphoprotein 1 on development of mammalian conceptuses. Mol Cell Endocrinol 354:22–33

Bérard J, Bee G (2010) Effects of dietary L-arginine supplementation to gilts during early gestation on foetal survival, growth and myofiber formation. Animal 4:1680–1687

Costa RP, Costa AS, Korzekwa AJ et al (2008) Actions of a nitric oxide donor on prostaglandin production and angiogenic activity in the equine endometrium. Reprod Fertil Dev 20:674–683

Dai ZL, Li XL, Xi PB et al (2012a) Regulatory role for L-arginine in the utilization of amino acids by pig small-intestinal bacteria. Amino Acids 43:233–244

Dai ZL, Li XL, Xi PB et al (2012b) Metabolism of select amino acids in bacteria from the pig small intestine. Amino Acids 42:1597–1608

Gao KG, Jiang ZY, Lin YC et al (2012) Wu G. Dietary L-arginine supplementation enhances placental growth and reproductive performance in sows. Amino Acids 42:2207–2214

Geng MM, Li TJ, Kong XF et al (2011) Reduced expression of intestinal N-acetylglutamate synthase in suckling piglets: a novel molecular mechanism for arginine as a nutritionally essential amino acid for neonates. Amino Acids 40:1513–1522

Go GW, Wu G, Silvey DT et al (2012) Lipid metabolism in pigs fed supplemental conjugated linoleic acid and/or dietary arginine. Amino Acids 43:1713–1726

Kim JY, Burghardt RC, Wu G et al (2011) Select nutrients in the ovine uterine lumen. VIII. Arginine stimulates proliferation of ovine trophectoderm cells through MTOR-RPS6 K-RPS6 signaling cascade and synthesis of nitric oxide and polyamines. Biol Reprod 84:70–78

Kim JY, Song GW, Wu G et al (2012) Functional roles of fructose. Proc Natl Acad Sci USA 109:E1619–E1628

Kong XF, Tan BE, Yin YL et al (2012) L-Arginine stimulates the mTOR signaling pathway and protein synthesis in porcine trophectoderm cells. J Nutr Biochem 23:1178–1183

Li XL (2011) Regulation of porcine conceptus survival and growth by L-arginine (Ph D Dissertation). Texas A&M University, College Station

Li X, Bazer FW, Johnson GA et al (2010) (2010), Dietary supplementation with 0.8% L-arginine between Days 0 and 25 of gestation reduces litter size in gilts. J Nutr 140:1111–1116

Li XL, Rezaei R, Li P et al (2011) Composition of amino acids in feed ingredients for animal diets. Amino Acids 40:1159–1168

Liu XD, Wu X, Yin YL et al (2012) Effects of dietary L-arginine or N-carbamylglutamate supplementation during late gestation of sows on the miR-15b/16, miR-221/222, VEGFA and eNOS expression in umbilical vein. Amino Acids 42:2111–2119

Mateo RD, Wu G, Bazer FW et al (2007) Dietary L-arginine supplementation enhances the reproductive performance of gilts. J Nutr 137:652–656

McKnight JR, Satterfield MC, Jobgen WS et al (2010) Beneficial effects of L-arginine on reducing obesity: potential mechanisms and important implications for human health. Amino Acids 39:349–357

National Research Council (NRC) (1998) Nutrient requirements of swine, 10th edn. National Academic Press, Washington, DC

Pope WF (1994) Embryonic mortality in swine. In: Zavy M, Geisert R (eds) Embryonic mortality in domestic species. CRC Press, Boca Raton, pp 53–77

Ramaekers P, Kemp B, van der Lende T (2006) Progenos in sows increases number of piglets born. J Anim Sci 84(Suppl1):394 (Abstract)

Ren W, Yin YL, Liu G et al (2012) Effect of dietary arginine supplementation on reproductive performance of mice with porcine circovirus type 2 infection. Amino Acids 42:2089–2094

Ren WK, Luo W, Wu MM et al (2013) Dietary L-glutamine supplementation improves pregnancy outcome in mice infected with type-2 porcine circovirus. Amino Acids 45:479–488

Reynolds LP, Borowicz PP, Vonnahme KA et al (2005) Placental angiogenesis in sheep models of compromised pregnancy. J Physiol 565:43–58

Rhoads JM, Wu G (2009) Glutamine, arginine, and leucine signaling in the intestine. Amino Acids 37:111–122

Roe JH (1934) A colorimetric method for the determination of fructose in blood and urine. J Biol Chem 107:15–22

Salvemini D, Misko TP, Masferrer JL et al (1993) Nitric oxide activates cyclooxygenase enzymes. Proc Natl Acad Sci USA 90:7240–7244

Satterfield MC, Dunlap KA, Keisler DH et al (2012) Arginine nutrition and fetal brown adipose tissue development in diet-induced obese sheep. Amino Acids 43:1593–1603

Satterfield MC, Dunlap KA, Keisler DH et al (2013) Arginine nutrition and fetal brown adipose tissue development in nutrient-restricted sheep. Amino Acids 45:489–499

Schoknecht PA, Pond WG, Mersmann HJ et al (1993) Protein restriction during pregnancy affects postnatal growth in swine progeny. J Nutr 123:1818–1825

Schoknecht PA, Newton GR, Weise DE et al (1994) Protein restriction in early pregnancy alters fetal and placental growth and allantoic fluid proteins in swine. Theriogenology 42:217–226

Smith MF, McIntush EW, Smith GW (1994) Mechanisms associated with corpus luteum development. J Anim Sci 72:1857–1872

Suryawan A, Davis TA (2011) Regulation of protein synthesis by amino acids in muscle of neonates. Front Biosci 16:1445–1460

Vonnahme KA, Ford SP (2004) Placental vascular endothelial growth factor receptor system mRNA expression in pigs selected for placental efficiency. J Physiol 554:194–201

Wu G (2013a) Functional amino acids in nutrition and health. Amino Acids 45:407–411

Wu G (2013b) Amino acids: biochemistry and nutrition. CRC Press, Boca Raton, Florida p 503

Wu G, Morris SM Jr (1998) Arginine metabolism: nitric oxide and beyond. Biochem J 336:1–17

Wu G, Bazer FW, Tuo W (1995) Developmental changes of free amino acid concentrations in fetal fluids of pigs. J Nutr 125:2859–2868

Wu G, Pond WG, Ott TL et al (1998a) Maternal dietary protein deficiency decreases amino acid concentrations in fetal plasma and allantoic fluid of pigs. J Nutr 128:894–902

Wu G, Pond WG, Flynn SP et al (1998b) Maternal dietary protein deficiency decreases nitric oxide synthase and ornithine decarboxylase activities in placenta and endometrium of pigs during early gestation. J Nutr 128:2395–2402

Wu G, Bazer FW, Wallace JM et al (2006) Board-invited review: intrauterine growth retardation: implications for the animal sciences. J Anim Sci 84:2316–2337

Wu G, Bazer FW, Davis TA et al (2009) Arginine metabolism and nutrition in growth, health and disease. Amino Acids 37:153–168

Wu G, Bazer FW, Burghardt RC et al (2010) Impacts of amino acid nutrition on pregnancy outcome in pigs: mechanisms and implications for swine production. J Anim Sci 88:E195–E204

Wu G, Bazer FW, Satterfield MC et al (2013a) Impacts of arginine nutrition on embryonic and fetal development in mammals. Amino Acids 45:241–256

Wu G, Wu ZL, Dai ZL et al (2013b) Dietary requirements of "nutritionally nonessential amino acids" by animals and humans. Amino Acids 44:1107–1113

Wu G, Bazer FW, Johnson GA et al (2013c) Maternal and fetal amino acid metabolism in gestating sows. Soc Reprod Fertil Suppl 68:185–198

Xi PB, Jiang ZY, Zheng CT et al (2011) Regulation of protein metabolism by glutamine: implications for nutrition and health. Front Biosci 16:578–597

Yao K, Yin YL, Chu W et al (2008) Dietary arginine supplementation increases mTOR signaling activity in skeletal muscle of neonatal pigs. J Nutr 138:867–872

Zeng X, Wang F, Fan X et al (2008) Dietary arginine supplementation during early pregnancy enhances embryonic survival in rats. J Nutr 138:1421–1425