NVEO
Natural Volatiles & Essential Oils

Effect of L-arginine supplementation on growth hormone and insulin-like factor 1 in pregnant mice

Ghassan Nissan[1] Yasmin L. Alsaadi[2]

1.,2 University of Baghdad/Iraq

Abstract

L- Arginine (L-Arg) is an essential amino acid in physiological fluids. The purpose of this study was to test the effect of dietary L-arginine supplementation in pregnant mice and its role in improving fetal growth. Sixty-six female mice with body weight (28-36 g) were divided into three groups (A, B, C) when pregnancy occurs. Each group was divided into 2 subgroups according to administration: 1-7 days and 1-15 days of pregnancy as followed as: group (**A**) 22 pregnant mice of this group gave normal saline (0.9% NaCl): 13 mice treated from (1-7 days) and 9 mice treated from (1-15 days). Group (**B**) 22 pregnant mice of this group gave 200mg of L-arginine /kg:13 mice treated from (1-7 days) and 9 mice treated from (1-15 days). Group (**C**) 22 pregnant mice of this group gave 400mg of L-arginine /kg:13 mice treated from (1-7 days) and 9 mice treated from (1-15 days). after birth, blood by direct cardiac puncture were collected. Data were analyzed shows that the numbers of total fetuses born did not differ (P=0.703) among control and L-arg. supplemented groups with 200 mg/kg and 400 mg/kg during the gestational period (1-7 days). also, non-significant differences (P=0.681) in the number of live foetuses among control and L-arg. supplemented groups with 200 mg/kg and 400 mg/kg during the gestational period (1-15 days). The weight of live fetuses was significant increased (P≤0.01) in L-arg. supplemented groups with 200 mg/kg and with 400 mg/kg when compared with that of the control group during the gestational period (1-7 days). Additionally, there was a significant increase (P< 0.01) in L-arg. supplemented groups with 200 mg/kg and 400 mg/kg when compared with the control group during the gestational period (1-15 days). Hormonal assays, there was a significant (P< 0.01) increase in IGF-1 level in L-arg. supplemented groups with 200 mg/kg and 400 mg/kg during a gestational period (1-7 days). Whereas during a gestational period (1-15 days) also the results showed that there was a significant (P≤0.01) increase in the mean of IGF-1 level in L-arg. supplemented groups with 200 mg/kg and 400 mg/kg in compared to the control group. The result showed that no significant (P=0.492) correlation in growth hormone concentration among the groups during a gestational period (1-7 days). While during a gestational period (1-15 days) there were significant (P≤0.05) differences between the mean value of growth hormone in the control group was found to be (12.47 ±0.08), in L-arg. supplemented groups with 200 mg/kg it was (18.21 ±3.90), and groups with 400 mg/kg was (13.78 ±0.37).

Key words: L-arginine, GH, IGF-1

Introduction

Over 700 amino acids can be found in nature; however, only 20 of them have been recognized as building blocks for proteins in cells (Wu, 2014). However, certain amino acids, such as arginine, methionine, and cysteine are precursors for other essential molecules in the immune defense, antioxidant system, cell signaling, and gene expression, and can act as regulators in the growth and development of the animals, being classified as functional amino acids (Castro, *et al*., 2020). Likewise, arginine (Arg) has been associated with the synthesis of protein and other metabolically-important molecules, such as creatine, nitric oxide, glutamate, polyamines, proline, and glutamine (Khajali, *et al*., 2010). Arginine plays a key role in pregnancy and fetal development. L-arginine specifically stimulates the secretion of growth hormone (GH) (Collier, *et al*., 2006), insulin (Umeda, *et al*., 2015), insulin-like growth factor 1 (IGF-1) (Kulkarni, *et al*., 2002), and other hormones. IGF-1 is an essential regulator of important processes of mammalian physiology, including cellular metabolism, proliferation, and growth (Pollak, 2008).

L-arginine has been reported to promote cellular and organismal growth and there is evidence that increased arginine intake increases the GH/IGF-1 release (Oh, *et al*., 2017). In mammalian fetus, L-

arginine is a crucial amino acid, and the conservation of arginine concentration is essential for most favourable fetal growth (Thureen, *et al*., 2002; Lassala, *et al*., 2010). The fetal development and survival in rats and gilts have improved by dietary supplementation of L-arginine (Lassala, *et al*., 2009; Greene, *et al*., 2012). the present study was designed to evaluate the role of L-arginine on growth hormone and insulin-like growth factor-1 in pregnant mice and its correlation with neonatal outcome.

Material and Methods

Healthy adult female mice were obtained from Al-Razi Center for research and production of diagnostic and medical tools, Ministry of Industry and Minerals, Baghdad-Iraq.

Virgin female mice (8-12 weeks) with weight range of 28-36 grams were used in this study. They were put in plastic cages with a metal network cover. These animals were kept under 20-25°C in an air-conditioned room and light/dark cycle of 12 hours daily.

The pregnant mice of the (A, B, C) groups were given dose orally by modified stomach tube and sacrificed at the end of the experiment. L-arginine was used as a dietary supplement by administering one millilitre of it by the rout of gavage in two concentrations (200 and 400) mg /kg of body weight of the mice by aiding a special needle.

Experimental Design:

Sixty-six female mice were divided into three groups (A, B, C) when pregnancy occurs. Each group was divided into 2 subgroups according to administration: 1-7 days and 1-15 days of pregnancy as follow as:

A- 22 pregnant mice of this group gave normal saline (0.9% NaCl): 13 mice treated from (1-7 days) and 9 mice treated from (1-15 days).

B- 22 pregnant mice of this group gave 200mg of L-arginine /kg:13 mice treated from (1-7 days) and 9 mice treated from (1-15 days).

C- 22 pregnant mice of this group gave 400mg of L-arginine /kg:13 mice treated from (1-7 days) and 9 mice treated from (1-15 days).

The blood samples were collected for analysis by direct cardiac puncture from Pregnant mice in each group after the birth.

This method is approached by separating the serum by centrifuge for the purpose of doing the biochemical tests of GH and IGF-1 by using kits to detect the effect of L-arginine on pregnant mice.

Statistical analysis

The Statistical Analysis System- SAS (2012) program was used to detect the effect of difference factors in study parameters. Least significant difference –LSD test (Analysis of Variation-ANOVA) was used to significant compare between means in this study.

Results and Discussion.

Experimental parameters

Number of live fetuses

The result shows the total number of live fetuses did not differ among control and L-arg. supplemented groups with 200 mg/kg and 400 mg/kg during gestational period (1-7 day) (**Table 3-1).**

Table 3-1: Number of live fetuses of the study groups treated with L-arg. during gestational period (1-7 day)

Study groups	Mean ± SE
control with normal saline	4.22 ±0.14
Treated with L-arg. 200 mg/kg	4.00 ±0.17
Treated with L-arg. 400 mg/kg	4.22 ±0.14
LSD value	1.667 NS
P-value	0.703
Non-Significant.	

Additionally, the current study showed that non-significant differences in number of live fetuses among arginine-supplemented groups with 200 mg/kg and 400 mg/kg in Comparison to control group during gestational period (1-15 day) (**Table 3-2).**

Table 3-2: Number of live fetuses of the study groups treated with L-arg. during gestational period (1-15 day)

Study groups	**Mean ± SE**
Control with normal saline	4.33 ±0.17
Treated with L-arg. 200 mg/kg	4.33 ±0.23
Treated with L-arg. 400 mg/kg	4.44 ±0.24
LSD value	2.174 NS
P-value	0.681
Non-Significant	

Results showed that no significant differences in number of live fetuses among control and L-arg. supplemented groups with 200 mg/kg and 400 mg/kg during gestational period (1-7 day) and (1-15 day). Our data are in accord with those of Bass, *et al*. (2017) and Mateo, *et al*. (2007) who found that

the number of piglets born did not vary between for arginine-supplemented gilts compared with gilts fed the control diet. While Li, *et al*. (2010) observed that number of live fetuses was reduced (P< 0.05) in gilts supplemented with 0.8% L-arginine compared with control gilts.
On the Contrary, Luise, *et al*. (2020) who reported that L-arg. ameliorated the number of total born piglets and tended to get better the number of totals born alive and to diminish dead piglets. Gonçalves, *et al.* 2016 mentioned in their study how the addition of L-arg. in the initial stage of gestation of the sow, has preferred embryonic survival, the number of births and birth weight. As well as Zeng, *et al*. (2008) and Al-Bayati, *et al*. (2014) who found that the total number of live-born mice was elevated in the arginine supplemented group compared with the control group. Also, another study found Compared with the control; arginine supplementation augmented the total number of piglets (Mateo, *et al*., 2007; Gao, *et al*., 2012).

Weight of live fetuses

Results of this study showed that there were significant (P≤0.01) differences in the weight of live fetuses in L-arg. supplemented groups with 200 mg/kg (1.509 ± 0.016 gm) and with 400 mg/kg (1.571 ± 0040 gm) when compared with that of the control group (1.370 ± 0019 gm) during gestational period (1-7 day), but no significant differences between two L-arg. supplemented groups (**Table 3-3)**.

Additionally, the mean value of weight of live fetuses in L-arg. supplemented group with 200 mg/kg was found to be (1.536 ± 0.028 gm), while in L-arg. supplemented groups with 400 mg/kg it was (1.436 ± 0.019 gm), and in the control group was (1.334 ± 0.017 gm) during gestational period (1-15 day). There were significant (P< 0.01) differences among the means of the three studied groups, as shown in (**Table 3-4).** Also, a result showed that period of treatment (1-7 day) with L-arg. (400 mg/kg) is preferable, which culminates in the heaviest weight at birth.

Table 3-3: Weight of live fetuses (gm) in the study groups treated with L-arg. during gestational period (1-7 day)

Study groups	Mean ± SE
Control with normal saline	1.370 ± 0019 b
Treated with 200 mg/kg	1.509 ± 0.016 a
Treated with 400 mg/kg	1.571 ± 0040 a
LSD value	0.0728 **
P-value	0.00826
** (P≤0.01)	

Table 3-4: Weight of live fetuses (gm) in the study groups treated with L-arg. during gestational period (1-15 day)

Study groups	Mean ± SE
Control with normal saline	1.334 ± 0.017 c
Treated with 200 mg/kg	1.536 ± 0.028 a
Treated with 400 mg/kg	1.436 ± 0.019 b
LSD value	0.0877 **
P-value	0.0094
** (P≤0.01)	

The present results were in agreement with previous studies done by (Mateo, *et al*., 2007; Greene, *et al*., 2012; Al-Bayati, *et al*., 2014) who referred that arginine supplementation raised the birth weight of piglets and mice. As well as other studies by (Gao, *et al*., 2012; Nuntapaitoon, *et al*., 2018) reported a considerable improvement in the weight of piglets at birth. De Boo, *et al*. (2005) showed that L-arginine infusion into animal throughout gestational period increased protein accumulation in fetus, consequently, increased the fetal weight as well as Wu, *et al*. (2013) who reported that dietary supplementation with L-arg. to gilts excesses the number of live-born piglets and birth weight. Alike results have been reported for gestating ewes and rats. So, enhancement of uterine in addition to placental growth and function through dietary L-arg. supplementation provides an effectual solution to amelioration embryonic and fetal survival and growth. Chen, *et al*. (2016) mentioned in their study that L-arg. considerably increased birth weight of IUGR fetuses. Also, Lassala, *et al*. (2011) confirmed that ewes given L- arginine had heavier lamb birth weight than control.

Si-
eroszewski et al. (2004) recently demonstrated durable im-
provement of foetal growth and increase of birth weight in
patients with intrauterine growth restriction treated with
low doses of L-arginine for 20 days, however patients with
preeclampsia were excluded from that study.

Si-
eroszewski et al. (2004) recently demonstrated durable im-
provement of foetal growth and increase of birth weight in
patients with intrauterine growth restriction treated with
low doses of L-arginine for 20 days, however patients with
preeclampsia were excluded from that study.
Si-
eroszewski et al. (2004) recently demonstrated durable im-
provement of foetal growth and increase of birth weight in
patients with intrauterine growth restriction treated with
low doses of L-arginine for 20 days, however patients with
preeclampsia were excluded from that study

However, our result disagree with (Zeng, *et al*., 2008; Gao, *et al*., 2012; Gonçalves, *et al*., 2016 ; Bass, *et al*., 2017; Luise, *et al*., 2020) who reported no significant differences in weight of live fetuses of sows between control and arginine-supplemented groups, Also Gui, *et al*. (2014) who found that the effect of L-arg. supplementation on increasing neonatal weight was not statistically significant. While Li, *et al*. (2010) observed that weight of live fetuses was reduced (P< 0.05) in gilts supplemented with 0.8% L-arginine compared with control gilts.

Hormonal assays

Estimation of insulin-like growth factor 1 (IGF-1)

At the present study, the mean of IGF-1 level in control group was (6.88 ± 1.01), while in L-arg. supplemented groups with 200 mg/kg it was (27.09 ± 2.14), and in the L-arg. supplemented groups with 400 mg/kg was (89.04 ± 13.69) during gestational period (1-7 day) (**Table 3-5)**. Whereas during gestational period (1-15 day) the mean value of IGF-1 level in control group was found to be (6.69 ± 0.50), while in L-arg. supplemented groups with 200 mg/kg it was (118.26 ± 3.78), and in the L-arg. supplemented groups with 400 mg/kg was (156.36 ± 17.70) (**Table 3-6)**. The results showed that there were significant (P≤0.01) differences in the mean of IGF-1 level among control groups and L-arg. supplemented groups with 200 mg/kg and 400 mg/kg during gestational periods (1-15 day) and (1-7 day).

Table 3-5: Levels of Insulin-like growth factor 1 (ng/ml) in the study groups treated with L-arg. during gestational period (1-7 day)

Study groups	Mean ± SE
control with normal saline	6.88 ± 1.01 c
Treated with 200 mg/kg	27.09 ± 2.14 b
Treated with 400 mg/kg	89.04 ± 13.69 a
LSD value	18.772 **
P-value	0.0001
** (P≤0.01).	

Table 3-6: Levels of Insulin-like growth factor 1 (ng/ml) in the study groups treated with L-arg. during gestational period (1-15 day)

Study groups	Mean ± SE
control with normal saline	6.69 ± 0.50 c
Treated with 200 mg/kg	118.26 ± 3.78 b

Treated with 400 mg/kg	156.36 ± 17.70 a
LSD value	32.994 **
P-value	0.0001
** (P≤0.01)	

These results were in agreement with Zeitoun, *et al*. (2016) referred in their studies on ewes that IGF-1 level was increased at high dose of L-arg. at late pregnancy revealed significant elevation in IGF-1 level. Guo, *et al*. (2016) found that dietary L-arg. supplementation increased concentration of IGF-1 of gilts. As well as Zhang, *et al*. (2016) who found that dietary L-arg. supplementation enhanced the concentration of IGF-1. A study by Lu, *et al.* (2006) on rats found a significant increase of IGF-1 than control as they administered the rats with 100 mg or 200 mg L-arginine. Sun, *et al*. (2018) suggested that maternal L-arg. supplementation in ewes was improved the level of insulin-like growth factor 1 and it is effective in enhancing fetal growth.

Tsugawa, *et al*. (2019) who referred that arginine was then administered to these mice. Plasma GH levels were elevated at 30 min. on the other hand; IGF-1 production demonstrated two peaks after L-arg. administration. The first peak appeared at 2 min, followed by a second peak at 120 min. The regulation of IGF-1 secretion occurred by mechanisms, first GH stimulates the translation of IGF-1 and elevated IGF-1 protein levels, leading to secretion of IGF-1. Demonstrating that arginine-induced GH secretion at 30 min induces the second peak of IGF-1 secretion at 120 min. Second, arginine-stimulated IGF-1 secretion occurred at 2 min. Osgerby, *et al*. (2002) who reported that Growth hormone tendency among gestational stage was not different as a result of L-arginine, so variations in the IGF cascade have a role in normalization of fetal growth. Si, *et al.* (2021) mentioned in their study that the IGF-1 level in the serum of low L-arg. (2.5 gm/d) sika deer was considerably higher than those in the serum of control and high L-arg. deer. Also Xu, *et al*. (2018) mentioned in their study that L-arg. significantly augmented IGF-1 expression and secretion under chronic caloric restriction conditions.

Clinical studies have revealed that levels of IGFs within the maternal circulation are also correlated with fetal growth highlighting the potential for maternal IGFs to have an influence on pregnancy outcome (Grissa, *et al*., 2010).

On the contrary, previous works have shown that no differences were observed for maternal IGF-1level between L-arg. supplemental group and control group during late pregnancy (Bass, *et al*., 2011; Bass, *et al*., 2017). da Silva, *et al*. (2014) demonstrated that There was no significant difference noticed in the level of IGF-1 between L-arg. supplemental and placebo groups. The dietary L-arg. supplementation did not manifest to induce the production of IGF-1. Moreover, Fayh, *et al*. (2007) who reported that subjects were supplemented with 7 gm of L-arg. orally for 7 days and noticed no significant alter in the levels of GH and IGF-1 at the end of the supplementation period in adults. Also, Corpas, *et al*. (1993) found no significant alters in the serum level of IGF-1 after oral L-arginine supplementation.

Estimation of growth hormone (GH)

In the current study, the mean of growth hormone in control group was (14.22 ±0.48), in L-arg. supplemented groups with 200 mg/kg was (15.60 ±1.78), and in L-arg. supplemented groups with 400 mg/kg was (15.04 ±0.97) during gestational period (1-7 day). The result showed that no significant correlation among the means of the three studied groups (**Table 3-7)**.

While during gestational period (1-15 day) the mean value of growth hormone in control group was found to be (12.47 ±0.08), in L-arg. supplemented groups with 200 mg/kg it was (18.21 ±3.90), and for the L-arg. supplemented groups with 400 mg/kg was (13.78 ±0.37). There were significant (P≤0.05) differences between the means of L-arg. supplemented groups with 200 mg/kg and L-arg. supplemented groups with 400 mg/kg also, control group (**Table 3-8)**.

Table 3-7: Levels of growth hormone (ng/ml) in the study groups treated with L-arg. during gestational period (1-7 day)

Study groups	Mean ± SE
Control with normal saline	14.22 ±0.48
Treated with 200 mg/kg	15.60 ±1.78
Treated with 400 mg/kg	15.04 ±0.97
LSD value	3.835 NS
P-value	0.492
Non-Significantly	

Table 3-8: Levels of growth hormone (ng/ml) in the study groups treated with L-arg. during gestational period (1-15 day)

Group (1-15 day)	Mean ± SE
Control with normal saline	12.47 ±0.08 b
Treated with 200 mg/kg	18.21 ±3.90 a
Treated with 400 mg/kg	13.78 ±0.37 b
LSD value	4.015 *
P-value	0.0488
* (P≤0.05)	

The current study revealed, although growth hormone level was elevated in L-arg. supplemented groups when compared with that of the control group during gestational period (1-7 day) but these differences was not statistically significant. As well, results showed that there are significant (P≤0.05) differences between the means of L-arg. supplemented groups with 200 mg/kg and L-arg. supplemented groups with 400 mg/kg as well, control group. So, it is preferable to administer a daily dose of L-arg. (200 mg/kg) at gestational period (1-15 day) (Figure 3-3).

According to the result during gestational period (1-7 day) this result agree with Sun, *et al*. (2018) suggested that maternal L-arg. supplementation in ewes has no effect on the concentrations of growth hormone, also (Hu, *et al*., (2015); Wu, *et al*., 2009) who had reported that no significant difference in the level of growth hormone in L-arginine supplemented group in a comparison to control group, as well as other study by Alawiy, *et al*. (2019) who referred that no significant difference in the level of growth hormone between the L-arg. and control groups. Yunta, *et al*., (2015) who found that no differences between L-arg. supplemented group and control group were found in growth hormone level in pregnant dairy heifers. Also, Zhang, *et al*. (2016) who referred that the supplementation of L-arginine had no effect on level of growth hormone in serum of pregnant ewes. Osgerby, *et al*. (2002) who reported that level of growth hormone among gestational stage was not different as a result of L-arginine supplementation in ovine.

According to the result during gestational period (1-15 day) this result agree with Oh, *et al*. (2017) who observed that L-arginine induces the expression of GH and IGF-1 genes expression in cultured GH3 pituitary epithelium and HepG2 hepatocytes respectively. So, the treatment with L-arginine significantly increased the secretion of GH and IGF-1 hormones in cultured cells after stimulation with L-arginine for 24 h., also Adriao, *et al*. (2004) mentioned in their in vitro studies, hemipituitaries or GH3 cells were incubated in 1 ml of suitable medium containing L-arg. (15 or 150 mg). The study demonstrated that arginine induced GH gene expression in hemipituitaries and GH3 cells. So that arginine stimulates GH gene expression in parallel to its recognized GH-releasing activity.

Anderson, *et al.* (2019) who detected that L-arginine is a functional amino acid that plays an essential role in the regulation of urea cycle, protein synthesis and hepatic detoxification. As well, Dietary L-Arginine supplementation elevates level of growth hormone in dairy cattle. McConell, (2007) who referred that L-arginine is related to numerous functions in the pregnant women. Dietary L-arginine supplementation administered intravenously was found to promoted the response of growth hormone and nitric oxide in a dose-dependent manner, from 6.0 to 30.0 gm. de Boo, *et al*. (2008) who reported that L-arginine can ameliorate the secretion of growth hormone releasing hormone, and consequently augment in growth hormone level influencing somatic growth. Tapiero, *et al*. (2002) referred in their studies that L-arg. is classified as an essential amino acid for mammals. L-arg. serves as a precursor for creatine, which plays an essential role in the arginine catabolism and for the synthesis of proteins. Through L-arg. ability to increase secretion of growth hormone it influences immune function. A study by (Chew, *et al*., 1984; Alba-Roth, *et al*., 1988) who reported that supply of arginine during gestation has effects on the secretion of growth hormone. Chew, *et al.* (1984) L-arginine was elevated the levels of growth hormone, prolactin, and insulin. Urea nitrogen also was increased in blood serum but not total protein. The secretory response of growth hormone, prolactin, and insulin to daily infusion of L-arginine during the entire prepartum period was not reduced. So, repeated arginine infusion in late-pregnant cows noticeably increased growth hormone, prolactin, and insulin

This finding is in contrast with results from some review studies by Zeitoun, *et al*. (2016) who reported that injecting arginine pregnant ewes led to a decrease in growth hormone concentration during pregnancy.

Conclusion

The following conclusion were noticed:

- dietary supplementation with L-arginine markedly enhanced the reproductive performance of mice by increasing fetal birth weight.
- supplementing arginine to the diet for female mice during gestation improve embryonic survival.
- The beneficial effects of arginine supplementation are associated with enhanced IGF-1 level.
- These support the notion that arginine plays a key role in the nutrition and physiology of pregnant mammals for optimal growth and survival of the embryo.

References

- Adrião, M.; Chrisman, C. J.; Bielavsky, M.; Olinto, S. C.; Shiraishi, E. M. and Nunes, M. T. (2004). Arginine increases growth hormone gene expression in rat pituitary and GH3 cells. Neuroendocrinology. 79(1): 26-33.
- Alawiy, I. Kh.; Mohammed, T.R. and Majeed, A.F. (2019) Effect of Arginine and Selenium with Vitamin E on WBC and the level of hormones in Iraqi ewes discharged. Al-Anbar Journal of Veterinary Sciences. 12(1): 6-12.
- Alba-Roth, J.; MÜLLER, O. A.; SCHOPOHL, J. and WERDER, K. V. (1988). Arginine stimulates growth hormone secretion by suppressing endogenous somatostatin secretion. The Journal of Clinical Endocrinology & Metabolism. 67(6): 1186-1189.
- Al-Bayati, Mohanad A.; Ahmad, Marawan A. and Khamas, Wael. (2014). The Potential Effect of L-arginine on Mice Placenta. Advances in Pharmacoepidemiology & Drug Safety. 3(2): 1-9.
- Anderson, S. J.; Côté, S. D.; Richard, J. H. and Shafer, A. B. (2019). Genomic architecture of artificially and sexually selected traits in a wild cervid. bioRxiv: 841528.
- Bass, B. E.; Bradley, C. L.; Johnson, Z. B.; Boyd, R. D.; Usry, J. L.; Maxwell, C. V. and Frank, J. W. (2011). Influence of dietary L-arginine supplementation to sows during late gestation on sow and litter performance during lactation. Res. Ser. 597: 151-155.
- Bass, B. E.; Bradley, C. L.; Johnson, Z. B.; Zier-Rush, C. E.; Boyd, R. D.; Usry, J. L.; Maxwell, C. V. and Frank, J. W. (2017). Influence of dietary -arginine supplementation of sows during late pregnancy on piglet birth weight and sow and litter performance during lactation. Journal of Animal Science. 95(1): 248-256.
- Castro, F. L. D. S. and Kim, W. K. (2020). Secondary Functions of Arginine and Sulfur Amino Acids in Poultry Health. Animals. 10(11): 2106.
- Chen, J.; Gong, X.; Chen, P.; Luo, K. and Zhang, X. (2016). Effect of L-arginine and sildenafil citrate on intrauterine growth restriction fetuses: a meta-analysis. BMC pregnancy and childbirth. 16(1): 1-7.
- Chew, B. P.; Eisenman, J. R. and Tanaka, T. S. (1984). Arginine infusion stimulates prolactin, growth hormone, insulin, and subsequent lactation in pregnant dairy cows. Journal of Dairy Science. 67(11): 2507-2518.

- Collier, S. R.; Collins, E. and Kanaley, J. A. (2006). Oral arginine attenuates the growth hormone response to resistance exercise. Journal of Applied Physiology. 101: 848-852.
- Corpas, E.; Blackman, M. R.; Roberson, R.; Scholfield, D. and Harman, S. M. (1993). Oral arginine-lysine does not increase growth hormone or insulin-like growth factor-I in old men. Journal of gerontology. 48(4): M128-M128.
- da Silva, Davi Vieira; Conte-Junior, Carlos Adam; Paschoalin, Vânia Margaret Flosi and Silveira, Alvares Thiago da. (2014). Hormonal response to L-arginine supplementation in physically active individuals. Food & Nutrition Research. 58(22569): 1-6.
- de Boo, H. A.; Eremia, S. C.; Bloomfield, F. H.; Oliver, M. H. and Harding, J. E. (2008). Treatment of intrauterine growth restriction with maternal growth hormone supplementation in sheep. American journal of obstetrics and gynecology. 199(5): 559-e1-559-e9.
- De Boo, H. A.; Van Zijl, P. L.; Smith, D. E.; Kulik, W.; Lafeber, H. N. and Harding, J. E. (2005). Arginine and mixed amino acids increase protein accretion in the growth-restricted and normal ovine fetus by different mechanisms. Pediatric research. 58(2): 270-277.
- Fayh, A. P.; Friedman, R.; Sapata, K. B. and Oliveira, A. R. D. (2007). Effect of L-arginine supplementation on secretion of human growth hormone and insulin-like growth factor in adults. Arquivos brasileiros de endocrinologia e metabologia. 51(4): 587-592.
- Gao, K.; Jiang, Z.; Lin, Y.; Zheng, C.; Zhou, G.; Chen, F.; Yang, L. and Wu, G. (2012). Dietary L-arginine supplementation enhances placental growth and reproductive performance in sows. Amino Acids. 42(6): 2207-2214.
- Gonçalves, M. A. D.; Gourley, K. M.; Dritz, S. S.; Tokach, M. D.; Bello, N. M.; DeRouchey, J. M.; Woodworth, J. C. and Goodband, R. D. (2016). Effects of amino acids and energy intake during late gestation of high-performing gilts and sows on litter and reproductive performance under commercial conditions. Journal of animal science. 94(5): 1993-2003.
- Greene, Jonathan M.; Dunaway, Chad W.; Bowers, Susan D.; Rude, Brain J.; Feugang, Jean M. and Ryan, Peter L. (2012). Dietary L-arginine supplementation during gestation in mice enhances reproductive performance and Vegfr2 transcription activity in the fetoplacental unit. The Journal of Nutrition. 142(3): 456-460.
- Grissa, O.; Yessoufou, A.; Mrisak, I.; Hichami, A.; Amoussou-Guenou, D.; Grissa, A.; Djrolo, F.; Moutairou, K.; Miled, A.; Khairi, H.; Zaouali, M.; Bougmiza, I.; Zbidi, A.; Tabka, Z. and Khan, N. A. (2010). Growth factor concentrations and their placental mRNA expression are modulated in gestational diabetes mellitus: possible interactions with macrosomia. BMC Pregnancy and Childbirth. 10(1): 1-10.
- Gui, S.; Jia, J.; Niu, X.; Bai, Y.; Zou, H.; Deng, J. and Zhou, R. (2014). Arginine supplementation for improving maternal and neonatal outcomes in hypertensive disorder of pregnancy: a systematic review. Journal of the Renin-Angiotensin-Aldosterone System. 15(1): 88-96.
- Guo, P.; Jiang, Z. Y.;Gao, K. G.; Wang, L.; Yang, X. F.; Hu, Y. J.; Zhang J. and Ma, X. Y. (2016). Low-level arginine supplementation (0.1%) of wheat-based diets in pregnancy increases the total and live-born litter sizes in gilts. Animal Production Science. 57(6): 1091-1096.
- Hu, S.; Li, X.; Rezaei, R.; Meininger, C. J.; McNeal, C. J. and Wu G. (2015). Safety of long-term dietary supplementation with L-arginine in pigs. Amino Acids. 47(5): 925-36.

- Khajali, F. and Wideman, R. F. (2010). Dietary arginine: metabolic, environmental, immunological and physiological interrelationships. World's Poultry Science Journal. 66(4): 751-766.
- Kulkarni, RN.; Holzenberger, M.; Shih, DQ.; Ozcan, U.; Stoffel, M.; Magnuson, M. A. and Kahn, C. R. (2002). β-cell–specific deletion of the Igf1 receptor leads to hyperinsulinemia and glucose intolerance but does not alter β-cell mass. Nature genetics. 31(1): 111-115.
- Lassala, A.; Bazer, F. W.; Cudd, T. A.; Datta, S.; Keisler, D. H.; Satterfield, M. C.; Spencer, T. E. and Wu, G. (2011). Parenteral administration of L-arginine enhances fetal survival and growth in sheep carrying multiple fetuses. The Journal of Nutrition. 141(5): 849-855.
- Lassala, Arantzatzu; Bazer, Fuller W.; Cudd, Timothy A.; Datta, Sujay; Keisler, Duane H.; Satterfield, M. Carey; Spencer, Thomas E. and Wu, Guoyao. (2010). Parenteral administration of L-arginine prevents fetal growth restriction in undernourished ewes. The Journal of Nutrition. 140(7): 1242-1248.
- Lassala, Arantzatzu; Bazer, Fuller W.; Cudd, Timothy A.; Li, Peng; Li, Xilong; Satterfield, M. Carey; Spencer, Thomas E. and Wu, Guoyao. (2009). Intravenous administration of L-citrulline to pregnant ewes is more effective than L-arginine for increasing arginine availability in the fetus. The Journal of Nutrition. 139(4): 660-665.
- Li, Xilong; Bazer, Fuller W.; Johnson, Gregory A.; Burghardt, Robert C.; Erikson, David W.; Frank, James W.; Spencer, Thomas E.; Shinzato, Izuru and Wu, Guoyao. (2010). Dietary supplementation with 0.8% L-arginine between days 0 and 25 of gestation reduces litter size in gilts. The Journal of Nutrition. 140(6): 1111-1116.
- Lu, Y.; Liu, X. M. and Li, S. Q. (2006). Effects of L-arginine on the expression of insulin-like growth factors and insulin-like growth factor binding protein 3 in rats with intrauterine growth retardation. Chinese Journal of Contemporary Pediatrics. 8(4): 319-322.
- Luise, D.; Bertocchi, M.; Bosi, P.; Correa, F.; Spinelli, E. and Trevisi, P. (2020). Contribution of L-Arginine supplementation during gestation on sow productive performance and on sow microbial faecal profile. Italian Journal of Animal Science. 19(1): 330-340.
- Mateo, Ronaldo D.; Wu, Guoyao; Bazer, Fuller W.; Park, Jun C.; Shinzato, Izuru and Kim, Sung Woo. (2007). Dietary L-arginine supplementation enhances the reproductive performance of gilts. The Journal of Nutrition. 137(3): 652-656.
- McConell, G. K. (2007). Effects of L-arginine supplementation on exercise metabolism. Current Opinion in Clinical Nutrition & Metabolic Care. 10(1): 46-51.
- Nuntapaitoon, M.; Muns, R.; Theil, P. K. and Tummaruk, P. (2018). L-arginine supplementation in sow diet during late gestation decrease stillborn piglet, increase piglet birth weight and increase immunoglobulin G concentration in colostrum. Theriogenology. 121: 27-34.
- Oh, H. S.; Oh, S. K.; Lee, J. S.; Wu, C. and Lee, S. J. (2017). Effects of l-arginine on growth hormone and insulin-like growth factor 1. Food science and biotechnology. 26(6): 1749-1754.
- Osgerby, J. C.; Wathes, D. C.; Howard, D. and Gadd, T. S. (2002). The effect of maternal undernutrition on ovine fetal growth. Journal of Endocrinology. 173(1): 131-142.
- Pollak, M. (2008). Insulin and insulin-like growth factor signalling in neoplasia. Nature Reviews Cancer. 8(12): 915-928.

- **SAS. 2012.** Statistical Analysis System, User's Guide. Statistical. Version 9.1th ed. SAS. Inst. Inc. Cary. N.C. USA.
- Si, H.; Liu, H.; Nan, W.; Li, G.; Li, Z. and Lou, Y. (2021). Effects of arginine supplementation on serum metabolites and the rumen bacterial community of sika deer (Cervus nippon). Frontiers in Veterinary Science. 8(630686): 1-9
- Sun, L.; Zhang, H.; Wang, Z.; Fan, Y.; Guo, Y. and Wang, F. (2018). Dietary rumen-protected arginine and N-carbamylglutamate supplementation enhances fetal growth in underfed ewes. Reproduction, Fertility and Development. 30(8): 1116-1127.
- Tapiero, H.; Mathe, G.; Couvreur, P. and Tew, K. D. (2002). I. Arginine. Biomedicine & pharmacotherapy. 56(9): 439-445.
- Thureen, Patti J.; Baron, Karen A.; Fennessey, Paul V. and Hay, William W. (2002). Ovine Placental and Fetal Arginine Metabolism at Normal and Increased Maternal Plasma Arginine Concentrations. Pediatric research. 51(4): 464-471.
- Tsugawa, Y.; Handa, H. and Imai, T. (2019). Arginine induces IGF-1 secretion from the endoplasmic reticulum. Biochemical and biophysical research communications. 514(4): 1128-1132.
- Umeda, M.; Hiramoto, M.; Watanabe, A.; Tsunoda, N. and Imai, T. (2015). Arginine-induced insulin secretion in endoplasmic reticulum. Biochemical and biophysical research communications. 466(4): 717-722.
- Wu, G. (2014). Dietary requirements of synthesizable amino acids by animals: a paradigm shift in protein nutrition. Journal of animal science and biotechnology. 5(1): 1-12.
- Wu, G.; Bazer, F. W.; Davis, T. A.; Kim, S. Woo; Li, Peng; Rhoads, Jon; Satterfield, Carey M.; Smith, Stephen B.; Spencer, Thomas E. and Yin, Y. (2009). Arginine metabolism and nutrition in growth, health and disease. Amino acids. 37(1): 153-168.
- Wu, Guoyao; Bazer, Fuller W.; Satterfield, M. Carey; Li, Xilong; Wang, Xiaoqiu; Johnson, Gregory A.; Burghardt, Robert C.; Dai, Zhaolai; Wang, Junjun and Wu, Zhenlong. (2013). Impacts of arginine nutrition on embryonic and fetal development in mammals. Amino Acids. 45(2): 241-256.
- Xu, J.; Zhu, C.; Zhang, M.; Tong, Q.; Wan, X.; iao, Z.; Cai, X.; Xu, Y.; Yuan, Y.; Wang, L.; Zhu, X.; Wang, S.; Gao, P.; Xi, Q.; Xu, Y.; Jiang, Q. and Shu, G. (2018). Arginine reverses growth hormone resistance through the inhibition of toll-like receptor 4-mediated inflammatory pathway. Metabolism. 79:10-23
- Yunta, C.; Vonnahme, K. A.; Mordhost, B. R.; Hallford, D. M.; Lemley, C. O.; Parys, C. and Bach, A. (2015) Arginine supplementation between 41 and 146 days of pregnancy reduces uterine blood flow in dairy heifers. Theriogenology. 84(1): 43-50.
- Zeitoun, M.; Al-Ghoneim, A.; Al-Sobayil, K. and Al-Dobaib, S. (2016). L-Arginine modulates maternal hormonal profiles and neonatal traits during two stages of pregnancy in sheep. Open Journal of Animal Sciences. 6(02): 95-104.
- Zeng, Xiangfang; Wang, Fenglai; Fan, Xia; Yang, Wenjun; Zhou, Bo; Li, Pengfei; Yin, Yulong; Wu, Guoyao and Wang, Junjun. (2008). Dietary arginine supplementation during early pregnancy enhances embryonic survival in rats. The Journal of Nutrition. 138(8): 1421-1425.
- Zeng, Xiangfang; Wang, Fenglai; Fan, Xia; Yang, Wenjun; Zhou, Bo; Li, Pengfei; Yin, Yulong; Wu, Guoyao and Wang, Junjun. (2008). Dietary arginine supplementation during early pregnancy enhances embryonic survival in rats. The Journal of Nutrition. 138(8): 1421-1425.

- Zhang, H.; Sun, L.; Wang, Z.; Deng, M.; Nie, H.; Zhang, G.; Ma, Tiewei and Wang, F. (2016). N-carbamylglutamate and L-arginine improved maternal and placental development in underfed ewes. Reproduction. 151(6): 623-635.
- Zhang, H.; Sun, L.W.; Wang, Z. Y.; Deng, M. T.; Zhang, G. M.; Guo, R. H.; Ma, T. W. and Wang, F. (2016). Dietary N-carbamylglutamate and rumen- protected L-arginine supplementation ameliorate fetal growth restriction in undernourished ewes. Journal of Animal Science. 94: 2072-2085.