Plant Archives Volume 20 No. 2, 2020 pp. 6485-6491 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

EFFECT OF USING N-CARBAMOYL GLUTAMATE (NCG) AS FEED ADDITIVE ON THE SEMEN QUALITY OF BULL

Adil Jabbar Atiyah[1], Hayder Al-Shanoon[1*], Kreem Iwaid Al-Badry[2], Faris Faisel Ibrahim[3] and Saadam Hussein Mahmoud[3]

[1*]Department of Veterinary Public Health, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq.
[2]Department of Surgery and Obstetrics, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq.
[3]Ministry of Agriculture, Department of Artificial Insemination Center, Baghdad, Iraq.

Abstract

N-carbamoyl glutamate (NCG) has been found to increase the arginine (ARG) level in the blood that is involved in many important biological activities. NCG exhibits lower ruminal degradation compared with ARG. This study aimed to evaluate the potential effects of NCG supplements as a feed additive on the semen quality of bull. The study was divided into two portions. In the first study, the semen was collected over six months (n = 6 Holstein bulls) with the aid of an artificial vagina. This period of experiment was divided into the pre-treatment period (control), treatment period (bull fed NCG 20g/day), and post-treatment period (Stop NCG treatment). In the first study, semen parameters were compared among different periods. The second study examined the semen parameters of the post-treatment group with those of bulls (n = 6) that were not treated with NCG previously (untreated group). From raw semen, the mass activity was determined. For other parameters, the semen was diluted with tris-based extender and evaluated for individual motility (after dilution, after cooling, and post-thawing). The results of the first study indicated that the semen parameters improved significantly ($P \leq 0.05$) in the post-treatment group compared with the control (pre-treatment) group. The second study revealed a significant ($P \leq 0.05$) improvement in mass activity and individual motility in the post-treatment group compared with the untreated group. These results suggest that NCG supplementation in routine feeding of bulls can improve the quality of semen.

Key words : N-carbamoyl glutamate, semen parameters, feed additive.

Introduction

The essential and non-essential amino acids have a crucial role in an animal's systemic level. Therefore, adding amino acids in animal feed is necessary for both maintenance and production (Guoyao Wu *et al.,* 2014). Thus, amino acids becomes an essential additive in farm animal feeding due to its positive effect on the animals' overall performance (Kellems & Church, 2002; Singh, 2015), especially in terms of reproductive efficiency (Mostafa *et al.,* 2014).

Arginine (ARG) is an amino acid that acts as a precursor for the synthesis of nitric oxide (NO) and polyamines. These components regulate the metabolic pathways that involve in the health, reproduction, growth and homeostasis of the animals (Palencia *et al.,* 2018; G. Wu, 2013). ARG is usually considered to be essential amino acid because ARG synthesis is not adequate to meet the animal requirement, specifically during the early stages of growth or for a high level of production (Guoyao Wu *et al.,* 2014). Studies also revealed that ARG used as a feed additive plays a critical role in improving male reproductive efficiency (Ahangar *et al.,* 2017; Chen *et al.,* 2018; Mendez & Hernandez, 1993).

In terms of semen quality, it has been directly proven that administration of ARG helps in the development of sperm, resulting in more ejaculate, higher sperm count, and better motility in men, boars, rabbits, fish, and roosters (Abbaspour *et al.,* 2019; Chen *et al.,* 2018; Kastelic, 2013; Radany & Atherton, 1981; Stanislavov & Rohdewald, 2014). Also, using ARG *in vitro* can improve semen motility and sperm count in different species (AL-

***Author for correspondence :** E-mail: headerfadel74@gmail.com

Ebady *et al.,* 2012; Keller & Polakoski, 1975; Maidin *et al.,* 2014). In bovine, most of the studies have focused on using ARG *in vitro*, not *in vivo*, on improving the quality of semen. However, unprotected ARG is not administered orally often to ruminants because of ARG is rapidly degraded in the rumen (Bahram Chacher *et al.,* 2012). Therefore, ARG reaches the small intestine with an insufficient amount where it is absorbed (Tauqir, 2016). Many studies were used rumen-protected ARG widely to increase the level of ARG in the blood of ruminants, such as cows, lambs, and ewes (Hassan *et al.,* 2011; Meyer *et al.,* 2018; H. Zhang *et al.,* 2016). However, using protected ARG seems to be uneconomical due to its high cost (B. Chacher *et al.,* 2013). N-carbamoyl glutamate (NCG), which is a structural analog of N-acetylglutamate, a cofactor of carbamoyl phosphate synthetase, has been used since 2014 to increase the level of ARG in the blood (B. Chacher *et al.,* 2014). NCG exhibited lower degradation in the rumen and had a lower cost than ARG (Bahram Chacher *et al.,* 2012). Moreover, NCG has been used to improve the reproductive efficiency and productivity of ruminant and non-ruminant animals (B. Chacher *et al.,* 2013; Chen *et al.,* 2018; B. Zhang *et al.,* 2014; H. Zhang *et al.,* 2016). However, no study has been conducted that shows the effect of NCG on the semen quality of bulls. This study aims to evaluate how dietary supplementation of NCG would affect semen quality.

Materials and Methods

1. Animal Management

This study was conducted at the artificial insemination center of Abou-Ghareeb, Agriculture Ministry (Baghdad-Iraq) from November 2018 to April 2019 on 12 Holstein bulls. Semen was routinely collected from all bulls weekly with the aid of an artificial vagina. In total, two hundred and ninety-six jaculates were evaluated during the study period. The average age of the bulls was 4–5 years. All bulls were managed under the same conditions in terms of feeding and watering throughout the study period.

2. Experiment Design

The work was divided into two studies. In the first study, the semen was collected from six bulls during the three periods: the pre-treatment period (from 2018/11/14 to 2019/1/10), which is considered as control; the treatment period (from 2019/1/14 to 2019/3/10), in which each bull was fed 20 g/day of NCG. The dose was determined based on browse study (B. Chacher *et al.,* 2014). The third period is the post-treatment (from 2019/3/11 to 2019/4/8; no NCG treatment). In the second study, the semen parameters of the post-treatment period were compared with those of the other six bulls that were not treated previously.

3. Semen Processing

Immediately after collection, all semen samples were placed in a water bath at 35°C to prevent temperature shock. The volume of the semen was measured using graduated test tubes. The semen concentration was evaluated using a spectrophotometer (Al-Badry *et al.,* 2016). Ejaculates with individual motility of spermatozoa of 50% or more were diluted in Tris–fructose–egg yolk–glycerol extender. Then, the diluted semen was placed in a water bath (37°C). For the freezing step, diluted semen was cooled by transferring it to the cold cabinet (5°C) for four hours. After the equilibration time, the diluted semen was packaged in straws (0.25 mL). Individual motility was assessed before freezing (cold semen). After freezing for 48 h in liquid nitrogen, the straws were thawed in a water bath 37°C for 30 seconds. The semen concentration was calculated using the spectrophotometer (Al-Badry *et al.,* 2016). The semen mass activity and individual sperm activity were evaluated by subjective measurement as follows.

3.1. Mass activity score analysis

The mass activity of spermatozoa was determined directly after semen collection. A drop of raw semen was poured on a worm slide (37°C) to observed under a microscope(magnification of 100×). The score of the mass activity was evaluated based on the following criteria: 0 = No wave, total immobility; 10 = No wave, individual movement; 20–40 = No wave, very slow movement; 45–65 = Wavy appearance, the slow amplitude of wave; 70–85 = Wavy appearance, rapid wave motion, no eddies; and 90–100 = Wavy appearance, rapid wave motion with eddies (Chemineau *et al.,* 1991).

3.2. Individual motility (percentage of motile spermatozoa) score analysis

Sperm motility analysis was conducted after diluting the raw semen with a 2.9% sodium citrate solution, with a ratio of 1:2 (semen/dilution). The mixture was examined under a phase-contrast light microscope (magnification of 400×) for each sample, and three fields were observed for every slide. Sperm motility was considered based on the percentage of sperm with the stander forward progressive movement. Any sperm with circling or oscillating movements were deemed to be immotile. The motility score was calculated based on the stander method described by Bearder et al. (Bearden & Fuquay, 2000).

4. Statistical Analysis

Data analysis was conducted using the SAS software

version 9.4 (SAS Institute Inc., Cary, NC, USA). One-way ANOVA was employed to observe the effect of NCG on the semen parameters in bull among the different periods (pre-treatment, treatment, and post-treatment). Pairwise Comparison of mean was performed, employing Tukey's multiple comparison test. The t-test was applied to analyze the data of semen from the post-treatment group vs. untreated group and pre-treatment group vs. treated group. Data are expressed as means ± SE; differences were regarded as significant at $P < 0.05$.

Results and Discussion

The sperm parameters of the first study are presented in table 1. Supplementation of NCG to bull significantly improves the sperm parameters except for volume in the post-treatment period compared with the pre-treatment period. The results of the pre-treatment periods varied sperm parameters did not significantly differ from those of the treatment period.

This study found that dietary NCG improves bull semen parameters. NCG's effect on reproductive efficiency has been studied mainly in females (Palencia *et al.,* 2018). To the best of our knowledge, there have been no previous studies documenting the impact of NCG on male reproductive efficiency, especially the semen parameters in any species. The positive effect of NCG on the semen parameters may be due to an increase in plasma ARG levels. Many studies found that the supplementation of NCG increases the endogenous synthesis of plasma ARG in piglets (Frank *et al.,* 2007), cows (B. Chacher *et al.,* 2014), and ewes (H. Zhang *et al.,* 2016). In many studies, ARG has been found to have a positive effect on sperm quality and activity. *In vitro*, ARG can stimulate sperm capacitation and acrosome reaction in bovine (O'Flaherty *et al.,* 2004) and sperm motility of human (Keller & Polakoski, 1975; Stanislavov & Rohdewald, 2014), bull (AL-Ebady *et al.,* 2012), and ram (Özer Kaya *et al.,* 2018). *In vivo,* supplementation of ARG, either orally or by injection, exhibited the same positive effect on sperm parameters. Intramuscular administration of L-arginine for seven weeks every 48 hours increased mass activity, motility, and concentration of ram sperm compared with control. Also, dietary L-arginine improved the semen quality of ram (Kaya *et al.,* 2019), boar (Chen *et al.,* 2018), men (Morgante *et al.,* 2010), fish (Kocabaº *et al.,* 2019; Pourkhazaei *et al.,* 2017), and roster(Ahangar *et al.,* 2017).

The results of this study agree with those of the reviews indicated above. The individual motility and the concentration of the sperm significantly increased after the administration of NCG to bulls compared with the pre-treatment group (Fig. 1). The ability of NCG to positively affect semen quality may arise from the nitric oxide (NO) pathway (Buzadzic *et al.,* 2015; Dixit & Parvizi, 2001; Miraglia *et al.,* 2011; Srivastava *et al.,* 2006); as ARG is the substrate for vascular NO formation (Palmer *et al.,* 1988; Rajapakse & Mattson, 2009). *Endogenous NO is beneficial to sperm motility (Lewis*

Table 1: Comparison of the semen parameters before, during, and after treatment with NCG (mean ± SE).

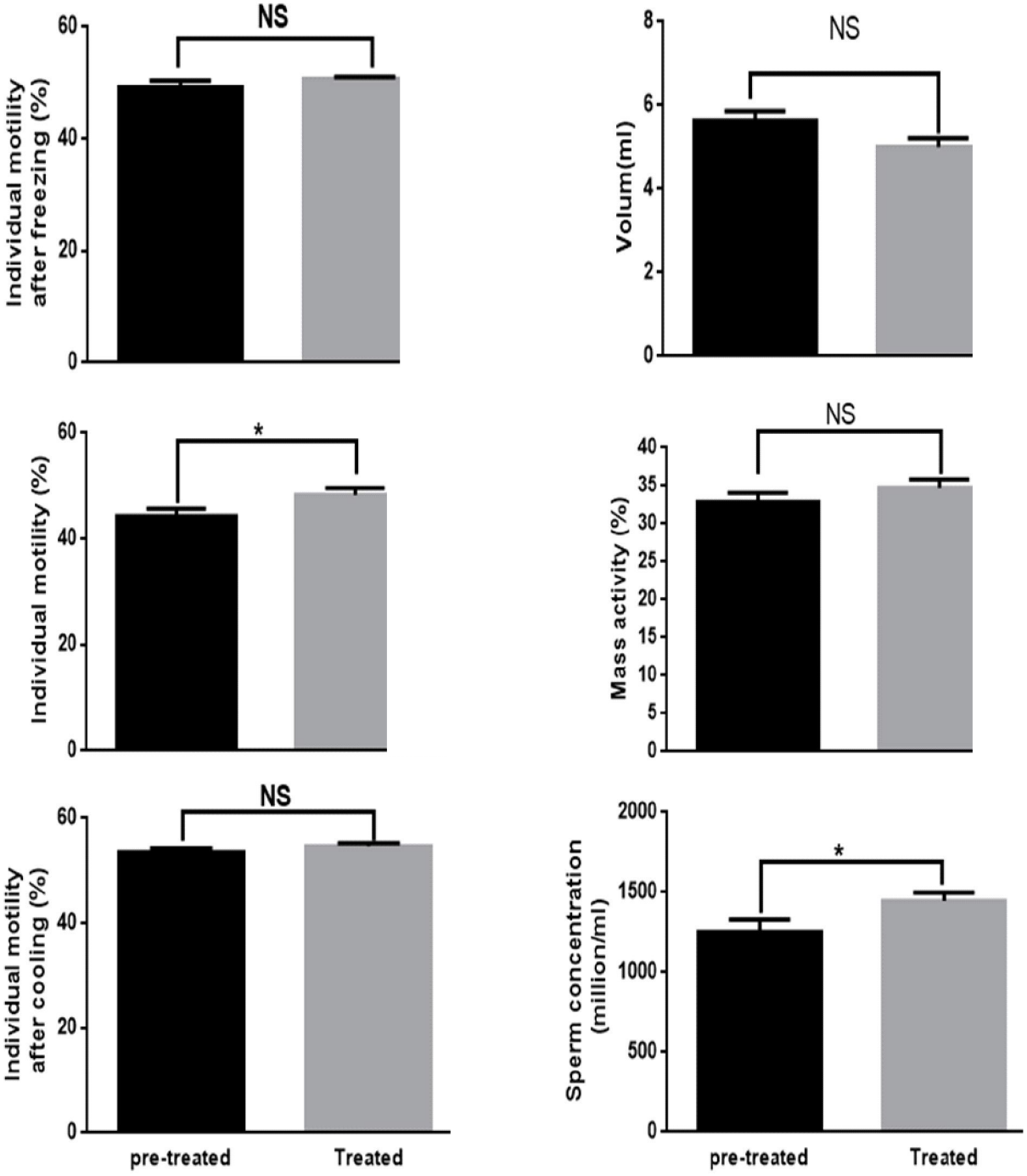
Sperm parameters	Pre-treatment	Treatment	Post-treatment	P-value
Volume (ml)	5.63 ± 0.23	5.01 ± 0.22	5.46 ± 0.30	0.09
Sperm concentration (million/ml)	1192 ± 89.55 A	1342 ± 63.22AB	1453 ± 50.57 B	0.03
Mass activity (%)	32.53 ± 1.08 A	34.75 ± 1.08 AB	37.63 ± 1.56 B	0.02
Individual motility (%)	44.51 ± 1.39 A	48.10 ± 1.40 AB	52.50 ± 2.14 B	e"0.01
Individual motility after cooling (%)	53.50 ± 0.82 A	54.72 ± 0.56 A	56.11 ± 0.82 B	0.001
Individual motility after freezing (%)	50.53 ± 0.36 A	50.69 ± 0.35 A	53.20 ± 0.86 B	0.03

NCG: N-Carbamoyl glutamate, [A, B,] significantly different among the periods $P < 0.05$.

Table 2: Comparison of the semen parameters between the untreated group and the post-treatment group.

Sperm parameters	Untreated group	Post-treatment group	P-value
Volume (ml)	5.84 ± 0.23	5.46 ± 0.30	0.45
Sperm concentration (million/ml)	1260 ± 67.60 A	1453 ± 50.57 B	0.01
Mass activity (%)	55.63 ± 1.83 A	37.63 ± 1.56 B	≥0.01
Individual motility (%)	38.13 ± 1.14 A	52.50 ± 2.14 B	≥0.01
Individual motility after cooling (%)	49.11 ± 2.37 A	56.11 ± 0.82 B	≥0.01
Individual motility after freezing (%)	42.22 ± 2.94 A	53.20 ± 0.86 B	0.005

[A, B] significantly different among the periods $P < 0.05$.



*Significantly different between groups $P < 0.05$. NS: Not significant.

Fig. 1: Comparison of the semen parameters between the pre-treatment group and treatment group.

et al., 1996). A study on frozen stallion sperm found a positive correlation between the NO level and sperm motility after thawing (Donnelly *et al*., 1997; Ortega Ferrusola *et al*., 2009). NO affects sperm motility *via* the activation of soluble guanylate cyclase, the subsequent synthesis of Cyclic guanosine monophosphate (cGMP), and the activation of cGMP-dependent protein kinases (Miraglia *et al.,* 2011). Also, NO increases the motility of spermatozoa by increasing energy production originating in the mitochondrial compartment (Otasevic *et al.,* 2013). However, many studies indicated that the effect of NO on sperm motility could be inconsistent, depending on the NO levels (Balercia *et al.,* 2004; Donnelly *et al.,* 1997; Weinberg *et al.,* 1995).

The exciting results of the post-treatment group exhibited a significant increase in all semen parameters,

except velum, compared with the pre-treatment group, despite the NCG treatment being halted in the post-treatment period. It is expected that long-term dietary supplementation of NCG (around three months) during the treatment period increases the ARG level leads to improve general health status, which may reflect positively on the results of the semen parameters. Other studies found ARG to be involved in many physiological functions and biological activities, such as antioxidant processes (Ma *et al.,* 2010; Wang *et al.,* 2019), maintenance of inter-organ metabolism and ammonia detoxification (Gebhardt *et al.,* 2003; G. Wu *et al.,* 2009), growth promotion (Jobgen *et al.,* 2006; G. Wu *et al.,* 2009; Yao *et al.,* 2011), and hormone secretion enhancement (Chew *et al.,* 1984; Oh *et al.,* 2017). ARG administration also increased the number of Sertoli cells in rabbit testes (Pitaloka, 2016). Sertoli cells have a significant role in regulating spermatogenesis and spermatozoa production (Griswold, 1998). The critical role of Sertoli cells results from providing structural support for sperm cells, regulation of nutrition development, and maintenance of a stable micro-environment for spermatogenesis (Griswold, 2018; Lee & Cheng, 2004). Sertoli cells are the main cellular targets for testosterone signaling, which is required to support male germ cell development and survival (Griswold, 1998). Recently, a study by Abbapsour *et al*., (2019) reported that dietary supplementation of ARG to old breed rosters increased testosterone concentration, testicular weight, tubular differentiation, spermatogenesis, and repopulation incident.

Based on the above studies, an increase in the number of Sertoli cells and improvement in the health status of the animal may support sperm production and quality after treatment (post-treatment). However, there is still concern that the improvement in sperm quality results from a seasonal effect. In Iraq, studies found that semen quality can change from month to month (Al-Ani & Abdulkareem, 2012; Al-Badry *et al.,* 2016; Mehdi *et al.,* 2012). In this study, individual motility improved significantly during March and April (the post-treatment period) compared with November–January (the pre-treatment period). Therefore, the second study was conducted to answer the following question: Is the improvement in semen quality caused by a seasonal change or by treatment with ARG? As such, the study investigated the quality of bull semen without NCG treatment compared with a post-treatment group (same period of the collection). The results of the second study table 2 indicate that treatment with NCG is the reason for the improvement in semen quality after the post-treatment group compared with a group of bulls that were not treated before. The mass activity, sperm concentration, individual motility, and individual motility after cooling and freezing increased significantly ($P < 0.01$) in the post-treatment group compared with the untreated group. The second study results reveal that the positive effect of NCG on semen quality can continue for a long time after treatment.

In summary, dietary NCG for bulls improves sperm motility and concentration when used as feed additive over long-term feeding.

Acknowledgments

The authors would introduce their thankful for workers in an artificial insemination center for collecting the data and their cooperation

References

Abbaspour, B., S.D. Sharifi, S. Ghazanfari, S. Honarbakhsh and A. Mohammadi-Sangcheshmeh (2019). The effect of l-arginine and flaxseed on plasma testosterone concentration, semen quality and some testicular histology parameters in old broiler breeder roosters. *Theriogenology,* **128:** 101-109. doi:10.1016/j.theriogenology.2019.01.034

Ahangar, M., S. Asadzadeh, V. Rezaeipour and A. Shahneh (2017). Effects of L-Arginine supplementation on semen quality, testosterone concentration and testes histological parameters of Ross 308 breeder roosters. *Asian Pacific Journal of Reproduction,* **6(3):** 133-135. doi:10.12980/apjr.6.20170307

Al-Ani, A.A. and T.A. Abdulkareem (2012). Influence of month on some sexual beviour and semen characteristics in Holstein- Friesian bulls. *Al-Anbar Journal of Veterinary Sciences,* **5(2):** 265-274.

Al-Badry, K.I., S.J. Zalala, F.F. Ibrahim and K.F. Hurish (2016). Monthly and Seasonality Variation in Freezability of Semen and Associations Between Freezability of Semen With Age and Testicular Dimension of Holstein Bulls Born in Iraq. *Indian Journal of Research,* **5:** 35-38.

AL-Ebady, A.S., S.O. Hussain, K.I. AL-Badry and B.A. Rajab (2012). Effect of adding arginine in different concentrations on some physical properties of poor motile bull sperms during different months. *Journal of Veterinary Medicine and Animal Health,* **4(9):** 130-135.

Balercia, G., S. Moretti, A. Vignini, M. Magagnini, F. Mantero, M. Boscaro and L. Mazzanti (2004). Role of nitric oxide concentrations on human sperm motility. *J. Androl,* **25(2):** 245-249.

Bearden, H.J. and J.W. Fuquay (2000). *Semen evaluation. In: Bearden H. J., Fuquay J.W., editors. Applied animal reproduction*. New Jersey: Prentice Hall, Upper Saddle River.

Buzadzic, B., M. Vucetic, A. Jankovic, A. Stancic, A. Korac, B. Korac and V. Otasevic (2015). New insights into male (in)fertility: the importance of NO. *Br. J. Pharmacol.,*

172(6): 1455-1467. doi:10.1111/bph.12675

Chacher, B., H. Liu, D. Wang and J. Liu (2013). Potential role of N-carbamoyl glutamate in biosynthesis of arginine and its significance in production of ruminant animals. *J. Anim. Sci. Biotechnol.,* **4(1):** 16. doi:10.1186/2049-1891-4-16

Chacher, B., D.M. Wang, H.Y. Liu and J.X. Liu (2012). Degradation of L-arginine and N-carbamoyl glutamate and their effect on rumen fermentation in vitro. *Italian Journal of Animal Science,* **11(4):** e68. doi:10.4081/ijas.2012.e68

Chacher, B., W. Zhu, J.A. Ye, D.M. Wang and J.X. Liu (2014). Effect of dietary N-carbamoylglutamate on milk production and nitrogen utilization in high-yielding dairy cows. *Journal of Dairy Science,* **97(4):** 2338-2345. doi:https://doi.org/10.3168/jds.2013-7330

Chemineau, P., Y. Cognine, Y. Guerin, P. Orgeure and J.C. Valtet (1991). Training Manual on Artificial Insemination in Sheep and Goats, Food and Agriculture Organization of the United Nations, Rome, FAO, Animal Production and Health Paper.

Chen, J.Q., Y.S. Li, Z.J. Li, H.X. Lu, P.Q. Zhu and C.M. Li (2018). Dietary l-arginine supplementation improves semen quality and libido of boars under high ambient temperature. *Animal,* **12(8):** 1611-1620. doi:10.1017/S1751731117003147

Chew, B.P., J.R. Eisenman and T.S. Tanaka (1984). Arginine infusion stimulates prolactin, growth hormone, insulin, and subsequent lactation in pregnant dairy cows. *J. Dairy Sci.,* **67(11):** 2507-2518. doi:10.3168/jds.S0022-0302(84)81607-0

Dixit, V.D. and N. Parvizi (2001). Nitric oxide and the control of reproduction. *Anim. Reprod. Sci.,* **65(1-2):** 1-16.

Donnelly, E.T., S.E. Lewis, W. Thompson and U. Chakravarthy (1997). Sperm nitric oxide and motility: the effects of nitric oxide synthase stimulation and inhibition. *Mol. Hum. Reprod.,* **3(9):** 755-762. doi:10.1093/molehr/3.9.755

Frank, J.W., J. Escobar, H.V. Nguyen, S.C. Jobgen, W.S. Jobgen, T.A. Davis and G. Wu (2007). Oral N-carbamylglutamate supplementation increases protein synthesis in skeletal muscle of piglets. *J. Nutr.,* **137(2):** 315-319. doi:10.1093/jn/137.2.315

Gebhardt, B., S. Vlaho, D. Fischer, A. Sewell and H. Bohles (2003). N-carbamylglutamate enhances ammonia detoxification in a patient with decompensated methylmalonic aciduria. *Mol. Genet. Metab.,* **79(4):** 303-304.

Griswold, M.D. (1998). The central role of Sertoli cells in spermatogenesis. *Semin. Cell. Dev. Biol.,* **9(4):** 411-416. doi:10.1006/scdb.1998.0203

Griswold, M.D. (2018). 50 years of spermatogenesis: Sertoli cells and their interactions with germ cells. *Biol. Reprod.,* **99(1):** 87-100. doi:10.1093/biolre/ioy027

Hassan, S., A.R. Ahmed, W. Al-Samaraae and A. Al-Badri (2011). Effects of rumen protected arginine supplementation on growth rate, rumen fermentation and blood biochemical of Awassi lambs. *KSÜ Doða Bilimleri Dergisi.,* **14(4):** 38-45.

Jobgen, W.S., S.K. Fried, W.J. Fu, C.J. Meininger and G. Wu (2006). Regulatory role for the arginine-nitric oxide pathway in metabolism of energy substrates. *J. Nutr. Biochem.,* **17(9):** 571-588. doi:10.1016/j.jnutbio.2005.12.001

Kastelic, J.P. (2013). Male involvement in fertility and factors affecting semen quality in bulls. *Animal Frontiers,* **3(4):** 20-25. doi:10.2527/af.2013-0029

Kaya, S.O., S. Gur and E. Kaya (2019). The effect of l-arginine on erection time, sperm quality and the seminal plasma arginase activity in rams. *Revue De Medecine Veterinaire,* **170(4-6):** 73-79.

Kellems, R.O. and D.C. Church (2002). *Livestock feeds and feeding*: Prentice Hall Upper Saddle River, NJ.

Keller, D.W. and K.L. Polakoski (1975). L-arginine stimulation of human sperm motility in vitro. *Biol. Reprod.,* **13(2):** 154-157. doi:10.1095/biolreprod13.2.154

Kocabas, M., F. Kutluyer, M. Erisir and F. Benzer (2019). L-Arginine improves sperm motility in threatened Coruh Trout Salmo coruhensis (Teleostei: Salmonidae). *Iranian Journal of Science and Technology, Transactions A: Science,* **43(4):** 1433-1437.

Lee, N.P. and C.Y. Cheng (2004). Adaptors, junction dynamics, and spermatogenesis. *Biol. Reprod.,* **71(2):** 392-404. doi:10.1095/biolreprod.104.027268

Lewis, S., E. Donnelly, E. Sterling, M. Kennedy, W. Thompson and U. Chakravarthy (1996). Nitric oxide synthase and nitrite production in human spermatozoa: evidence that endogenous nitric oxide is beneficial to sperm motility. *Molecular Human Reproduction,* **2:** 873-878.

Ma, X., Y. Lin, Z. Jiang, C. Zheng, G. Zhou, D. Yu and F. Chen (2010). Dietary arginine supplementation enhances antioxidative capacity and improves meat quality of finishing pigs. *Amino Acids,* **38(1):** 95-102.

Maidin, M.S., N.F. Adanan, M.T. Aminudin and A. Tawang (2014). In Vitro Supplements Improves Motility and Progressive Score of Spermatozoa in Jermasia Goats. *APCBEE Procedia,* **8:** 329-333. doi:https://doi.org/10.1016/j.apcbee.2014.03.049

Mehdi, M., A.L. Sahaf and N.S. Ibrahim (2012). Monthly changes in testes and epididymis measurements with some semen characteristics of tail epididymis for Iraqi buffalo *The Iraqi Journal of Veterinary Medicine,* **36(2):** 204-208.

Mendez, J.D. and M.P. Hernandez (1993). [Effect of L-arginine and polyamines on sperm motility]. *Ginecol. Obstet Mex,* **61:** 229-234.

Meyer, A.M., S.I. Klein, M. Kapphahn, D.V. Dhuyvetter, R.E. Musser and J.S. Caton (2018). Effects of rumen-protected arginine supplementation and arginine-HCl injection on site and extent of digestion and small intestinal amino acid disappearance in forage-fed steers1. *Translational Animal Science,* **2(2):** 205-215. doi:10.1093/tas/txy007

Miraglia, E., F. De Angelis, E. Gazzano, H. Hassanpour, A. Bertagna, E. Aldieri and D. Ghigo (2011). Nitric oxide stimulates human sperm motility via activation of the cyclic

GMP/protein kinase G signalling pathway. *Reproduction,* **141(1):** 47-54. doi:10.1530/REP-10-0151

Morgante, G., V. Scolaro, C. Tosti, A. Di Sabatino, P. Piomboni and V. De Leo (2010). [Treatment with carnitine, acetyl carnitine, L-arginine and ginseng improves sperm motility and sexual health in men with asthenopermia]. *Minerva Urol. Nefrol.,* **62(3):** 213-218.

Mostafa, T., F. Elsayed, M. Ahmed and M. Elkholany (2014). Effect of using some feed additives (tw-probiotics) in dairy cow rations on production and reproductive performance. *Egyptian J. Anim. Prod.,* **51(1):** 1-11.

O'Flaherty, C., P. Rodriguez and S. Srivastava (2004). L-arginine promotes capacitation and acrosome reaction in cryopreserved bovine spermatozoa. *Biochim. Biophys. Acta,* **1674(2):** 215-221. doi:10.1016/j.bbagen.2004.06.020

Oh, H.S., S.K. Oh, J.S. Lee, C. Wu and S.J. Lee (2017). Effects of l-arginine on growth hormone and insulin-like growth factor 1. *Food Sci. Biotechnol.,* **26(6):** 1749-1754. doi:10.1007/s10068-017-0236-6

Ortega Ferrusola, C., L. Gonzalez Fernandez, B. Macias Garcia, C. Salazar-Sandoval, A. Morillo Rodriguez, H. Rodríguez Martinez and F. Pena (2009). Effect of cryopreservation on nitric oxide production by stallion spermatozoa. *Biology of reproduction,* **81(6):** 1106-1111.

Otasevic, V., A. Korac, M. Vucetic, B. Macanovic, E. Garalejic, I. Ivanovic-Burmazovic and B. Korac (2013). Is manganese (II) pentaazamacrocyclic superoxide dismutase mimic beneficial for human sperm mitochondria function and motility? *Antioxid Redox Signal,* **18(2):** 170-178. doi:10.1089/ars.2012.4684

Özer Kaya, S., S. Gür and E. Kaya (2018). Effect of l arginine addition on long term storability of ram semen. *Andrologia,* **50(4):** e12945.

Palencia, J.Y.P., A. Saraiva, M.L.T. Abreu, M.G. Zangeronimo, A.P. Schinckel and C.A. Pospissil Garbossa (2018). Effectiveness of citrulline and N-carbamoyl glutamate as arginine precursors on reproductive performance in mammals: A systematic review. *PLoS One,* **13(12):** e0209569. doi:10.1371/journal.pone.0209569

Palmer, R.M., D.S. Ashton and S. Moncada (1988). Vascular endothelial cells synthesize nitric oxide from L-arginine. *Nature,* **333(6174):** 664-666. doi:10.1038/333664a0

Pitaloka, H.T. (2016). *The Effect Oral Administration Of L-Arginine On Spermatogenesis And Sertoli Cells Of Rabbits Testes (Oryctolagus cuniculus)*. (Ms.c Master Thesis), Agriculture University Surabaya. Retrieved from https://www.semanticscholar.org/paper/The-effect-oral-administration-of-L-Arginine-on-and-Pitaloka/08fbe036207ad7aae730058c9426da005dbb7285

Pourkhazaei, F., E. Ebrahimi and A. Ghaedi (2017). Arginine effects on biochemical composition of sperm in rainbow trout, Oncorhynchus mykiss. *Aquaculture research,* **48(7):** 3464-3471.

Radany, E.W. and R.W. Atherton (1981). Arginine induced stimulation of rabbit sperm motility. *Arch. Androl., 7***(4)**: 351-355.

Rajapakse, N.W. and D.L. Mattson (2009). Role of L-arginine in nitric oxide production in health and hypertension. *Clin. Exp. Pharmacol. Physiol.,* **36(3):** 249-255. doi:10.1111/j.1440-1681.2008.05123.x

Singh, P. (2015). *Animal Feed Additives* (P. K. Singh, K. K. Chandramoni, & S. Kumar Eds. Vol. 1). New Delhi - 110034, India: New India Publishing Agency.

Srivastava, S., P. Desai, E. Coutinho and G. Govil (2006). Mechanism of action of L-arginine on the vitality of spermatozoa is primarily through increased biosynthesis of nitric oxide. *Biol. Reprod.,* **74(5):** 954-958. doi:10.1095/biolreprod.105.046896

Stanislavov, R. and P. Rohdewald (2014). Sperm quality in men is improved by supplementation with a combination of L-arginine, L-citrullin, roburins and Pycnogenol(R). *Minerva Urol. Nefrol.,* **66(4):** 217-223.

Tauqir, N.A. (2016). Absorption and Transportation of Amino acids in animals: A Review. *Journal of Environmental and Agricultural Sciences,* **9:** 96-109.

Wang, L., J. Li, C. Wang, Z. Zhao, L. Luo, X. Du and Q. Xu (2019). Effect of N-carbamoylglutamate supplementation on the growth performance, antioxidant status and immune response of mirror carp (Cyprinus carpio) fed an arginine-deficient diet. *Fish Shellfish Immunol.,* **84:** 280-289. doi:10.1016/j.fsi.2018.10.001

Weinberg, J.B., E. Doty, J. Bonaventura and A.F. Haney (1995). Nitric oxide inhibition of human sperm motility. *Fertil. Steril.,* **64(2):** 408-413. doi:10.1016/s0015-0282(16)57743-7

Wu, G. (2013). Functional amino acids in nutrition and health. *Amino Acids,* **45(3):** 407-411. doi:10.1007/s00726-013-1500-6

Wu, G., F.W. Bazer, Z. Dai, D. Li, J. Wang and Z. Wu (2014). Amino Acid Nutrition in Animals: Protein Synthesis and Beyond. *Annual Review of Animal Biosciences,* **2(1):** 387-417. doi:10.1146/annurev-animal-022513-114113

Wu, G., F.W. Bazer, T.A. Davis, S.W. Kim, P. Li, J. Marc Rhoads and Y. Yin (2009). Arginine metabolism and nutrition in growth, health and disease. *Amino Acids,* **37(1):** 153-168. doi:10.1007/s00726-008-0210-y

Yao, K., S. Guan, T. Li, R. Huang, G. Wu, Z. Ruan and Y. Yin (2011). Dietary L-arginine supplementation enhances intestinal development and expression of vascular endothelial growth factor in weanling piglets. *Br. J. Nutr.,* **105(5):** 703-709. doi:10.1017/S000711451000365X

Zhang, B., L.Q. Che, Y. Lin, Y. Zhuo, Z.F. Fang, S.Y. Xu and D. Wu (2014). Effect of dietary N-carbamylglutamate levels on reproductive performance of gilts. *Reprod. Domest. Anim.,* **49(5):** 740-745. doi:10.1111/rda.12358

Zhang, H., L.W. Sun, Z.Y. Wang, M.T. Deng, G.M. Zhang, R.H. Guo and F. Wang (2016). Dietary -carbamylglutamate and rumen-protected -arginine supplementation ameliorate fetal growth restriction in undernourished ewes. *J. Anim. Sci.,* **94(5):** 2072-2085. doi:10.2527/jas.2015-9587