Vol. 19, No. 1, Spring, 2023, 83-89 *Original Article*
DOI: 10.22055/IVJ.2022.360735.2520
DOR: 20.1001.1.17356873.1402.19.1.9.4
Journal Homepage: http://www.ivj.ir/

The study of fat, Protein, and production levels of milk in Holstein dairy cows treated with arginine

Ali Tirgari[1], Majid Mohammadsadegh[2*], Morteza Gorjidoz[3], Ali Afshar Bahrabad[4] and Nima Farzaneh[5]

[1] DVM Graduated, Faculty of Veterinary Medicine, Garmsar Branch, Islamic Azad University, Garmsar, Iran
[2] Associate Professor, Department of Clinical Sciences, Faculty of Veterinary Medicine, Garmsar Branch, Islamic Azad University, Garmsar, Iran
[3] Assistant Professor, Department of Clinical Sciences, Faculty of Veterinary Medicine, Garmsar Branch, Islamic Azad University, Garmsar, Iran
[4] DVSc Graduate, Department of Theriogenology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran
5 Professor, Department of Clinical Sciences, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran

Received: 27.10.2022 Accepted: 30.11.2022

Abstract

In this study, 73 non-pregnant and clinically healthy and lactating Holstein cows without any clinical signs, and with a mean parity of 3.2 ± 1.4, days in milk at the beginning of study 110 ± 20 days, body condition scour (BCS) of about 3.2 ± 0.3, and milk production rate of 48± 10 kg were selected and randomly placed in two treatment and control groups. In the treatment group (n=36), arginine (155 µmol / kg body weight) was injected once every 8 hours a day for 6 days, and in the control group (n=37) saline solution (0.11 ml/kg body weight, once every 8 hours a day) was injected for 6 days. The results showed that there was no difference between the treatment and the controle group in terms of milk production (38.5 and 36 kg, P=0.3), fat (3.7 and 3.8%, P=0.8) and protein (3.1 and 3.1%, P=0.5) levels. It was concluded from this study that the use of arginine after the peak of milk production could not increase the fat, protein, and production levels of milk.

Keywords: Arginine, Amino Acid, Holstein Cow, Milk Production, Milk Fat and protein

Introduction

Many hormonal and non-hormonal products have been used to increase the milk production levels before or after the peak of milk production in dairy cows. Abomasal infusions of protein increased milk production via the changes in plasma concentrations of galactopoietic hormones (Clark, 1975). Growth hormone increased milk protein concentration and milk production levels (Burton et al, 1994) in mild and/or late-lactation in ewes (Chiofalo et al, 1999; Sallam et al, 2005) goats (Disenhaus et al, 1995) and cattle (Hayashi et al, 2009). Thyroid hormones or iodinated casein (thyroprotein) have been used toincrease milk production (Squires, 2003), but any net benefit has not been encountered.

* **Corresponding Author**: Majid Mohammadsadegh, Associate Professor, Department of Clinical Sciences, Faculty of Veterinary Medicine, Garmsar Branch, Islamic Azad University, Garmsar, Iran
E-mail: Majid.Mohammadsadegh@iau.ac.ir

Arginine is a conditionally essential amino acid that enhances casein synthesis in bovine mammary epithelial cells, so it should be able to enhance milk production (Wang et al, 2014). On the other hand, intravenous injection of arginine increases plasma concentrations of somatotropin (bST), nitric oxide (NO) (Mepham, 1982; Moncadaand and Higgs, 1993), insulin, prolactin, and placental lactogen in plasma of ruminants (Hertelendy et al, 1968; Chew et al, 1984; Hertelendy et al, 1969; Hertelendy et al, 1970; McAtee, 1971; McAtee and Trenkle, 1971; Davis, 1972). On the other hand, growth hormone or bovone somatotopin (bST) promotes some degrees of insulin resistance, lipolysis and β-hydroxy botyric ascid (BHBA), non esteified fat ascid (NEFA),oxidative stress (Zheng et al, 2018) and reduced glucose consumption (Azarbayejani and Mohammadsadegh, 2021; Oliveira et al, 2016), and total antioxidant capacity (TAC)(Zheng et al, 2018). The last findings probably show the reason for the decrease in milk in some studies following the use of arginine.

This article aimed to study the quantitative and qualitative change in the milk of Holstein cows after 6 days of intravenous injection of arginine at 110 days after calving.

Materials and method

Farms and animals' selection

Two dairy herds with about 3000 milking cows (HF=100%) and the same management around Varamin city in Tehran province were selected. The study was conducted from December 2018 (cold month in the region) to May 2019 (temperate month in the region) during 7 months. The cattle corral pen was a free stall, and the diet was adjusted by TMR twice a day based on the National Research council (NRC 2001). The average annual milk was 41 kg, and the animals were milked three times a day.To provide the required livestock in the study, 73 non-pregnant and clinically healthy and lactating Holstein cows without any clinical signs, and with a mean parity of 3.2 ± 1.4, days in milk at the beginning of the study 110 ± 20 days, BCS of about 3.2 ± 0.3 and milk production rate of 48± 10 kg were selected and randomly devided into two experimental and control groups.

Since the effect of arginine injection on the milk production before (Ding et al. 2019) and during the peak of lactation (Chew et al. 1984) was previously investigated, its effect after the peak of lactation and in the period carried out in this article was planned.

Animal groups and treatments

In the experimental group (n=36 cows), arginine (Arginine HCl, Merk co. Germany) was intravenously injected into jugular vein (155 μmol / kg body weight, equivalent to 0.032 gr/kg) once every 8 hours a day for 6 days based on Lassala et al. 2009; however, in the control group (n=37 cows) saline solution (daily 0.11 ml/kg body weight, every 8 hours) was injected once every 8 hours a day for 6 days as a placebo.

Blood and milk samplings

Milk samples were taken before and after 8 hours of the last arginine injection.

Before and after the arginine injection period, and daily milk production was estimated from all animals and milk samples were prepared and sent to the laboratory to measure the amount of fat and protein.

Blood samples were taken from the Coccidia vein and were prepared in 9 ml vacuum silicone tubes. Then the samples were placed in the environmental condition for one hour and then, using a centrifuge at a speed of 3000 rpm for 10 minutes, and their serum was isolated and placed at -70 °C. A refrigerator was used to carry the sample to the laboratory with a temperature of -4 °C so that the samples would not be

frozen and heat stress would not damage the samples.

Laboratory tests

Nitric oxide (ELISA 96 NATRIX diagnostic kit of Navand Salamat co., IRAN; with product code 15042NS), total antioxidant capacity (ELISA 96 diagnostic kit NAXIFER test of Navand Salamat co., IRAN; with product code 15012NS), Non esterified fatty acid (ELISA 96 RANBUT test kit by RANDOX English co., IRAN; with product code RX MONZA FA - 115), and beta-hydroxybutyric acid (ELISA 96 RANBUT test kit by RANDOX English co., IRAN; with product code RX MONAZA RB - 1007) were measured in serum samples in the laboratory.

Definition of research variables

In this study, arginine treatment was considered as an independent variable, the level of fat, protein, and production of milk as the dependent variable, and parity, days in milk, and BCS as confounding variables.Since the use of arginine could also increase the serum concentration of NEFA, bHBA, TAC, and NO, recent variables were also considered as under monitoring dependent variables.

Statistical analysis of data

In comparing statistical analysis of quantitative data such as fat, protein and the levels of milk production, serum concentrations of nitric oxide, total antioxidant capacity, NEFA and bHBA, first Shapiro-Wilk test and Kolmogorov-Smirnov test were used to check the normality of the distribution. Quantitative normal data were compared with the Student T-test and abnormal data were compared with Wilcoxon-rank-Sign and Mann-Whitney-U test. The 95% statistical confidence interval and the probability of type 1 error or significance level was considered 0.05. IBM SPSS version 24 (2018) and MEDICAL version 13 (2015) Software were used in data analysis.

Results

Shapiro-Wilk and Kolmogorov-Smirnov tests showed that none of the measurable research data had a normal distribution.The median milk production after the study was 38.5 kg in the experimental, and 36 kg in the control group (P = 0.3) (Table 3-1). The median milk production before the study (P=0.70) and also the difference in milk production (P=0.80) before and after injection in the two groups were not significantly different (Table 3-1). The median milk fat content after the study was 3.8% in the control group and 3.7% in the experimental group (P = 0.8) (Table 3-1). The median milk protein after the study in the control group was 1 3. and in the experimental group was 3.1% (P = 0.5) (Table 3-1). The median bHBA was 0.32 mg in the control, and 0.33 mg /ml in the experimental group (P = 0.2) (Table 2-3).The median NEFA was 0.2 mg in the control and 0.3 mg / ml in the experimental group (p = 0.13).The median NO was 1.3 in the control, and 2.4 mg /ml in the experimental group (p = 0.1).The median TAC was 0.32 in the control and 0.27 mg /ml in the experimental group (p = 0.001).

In comparison to confounder variables, the median age in the control was 1690 and in the experimental group was 1676 days (P = 0.37). The median parity in control was 3 and in the experimental group was 2.5 (P = 0.16). The median BCS in both groups was 3.2 (P = 0.8). The median interval between parturition and the beginning of the study was 221 days in the control and 211 days in the experimental group (P = 0.1).The median milk production before the start of the study was 44.7 kg in the control group and 40.7 kg in the experimental group (P = 0.7). The median amount of peak milk production before the start of the study in the control was 49.2 kg and in the experimental group it was 47.5 kg (P = 0.9). The median milk somatic cell count in the control was 48,000, and in the experimental group 95,000 (P = 0.00); however, both were at the normal range.

Table 1. Comparison of milk, fat, and protein production before and after the study between the control and experimental groups

Data	Groups								P=
	control				Arginine treated				
	Mean	SD	Median	95% CI	Mean	SD	Median	95% CI	
Milk (kg)after study	36.5	10.9	36	31.1 - 40.7	36.5	37.9	5.6	38.5	0.3
Milk fat (%)	3.6	0.5	3.8	3.4 - 3.8	3.6	1	3.7	3.6 - 3.9	0.8
Milk pro (%)	3.1	0.2	3.1	3.0 - 3.2	3.1	0.3	3.1	3.7 - 3.2	0.5
Milk (kg) before study	42.1	5.2	44.7	38.20- 46.	41.7	8	40.8	38.4 - 44.2	0.7
Milk difference	-4.16	3.3	-4.7	-6.6 - -2.9	-5.3	6.8	-3.7	-5.5 - -1.6	0.8

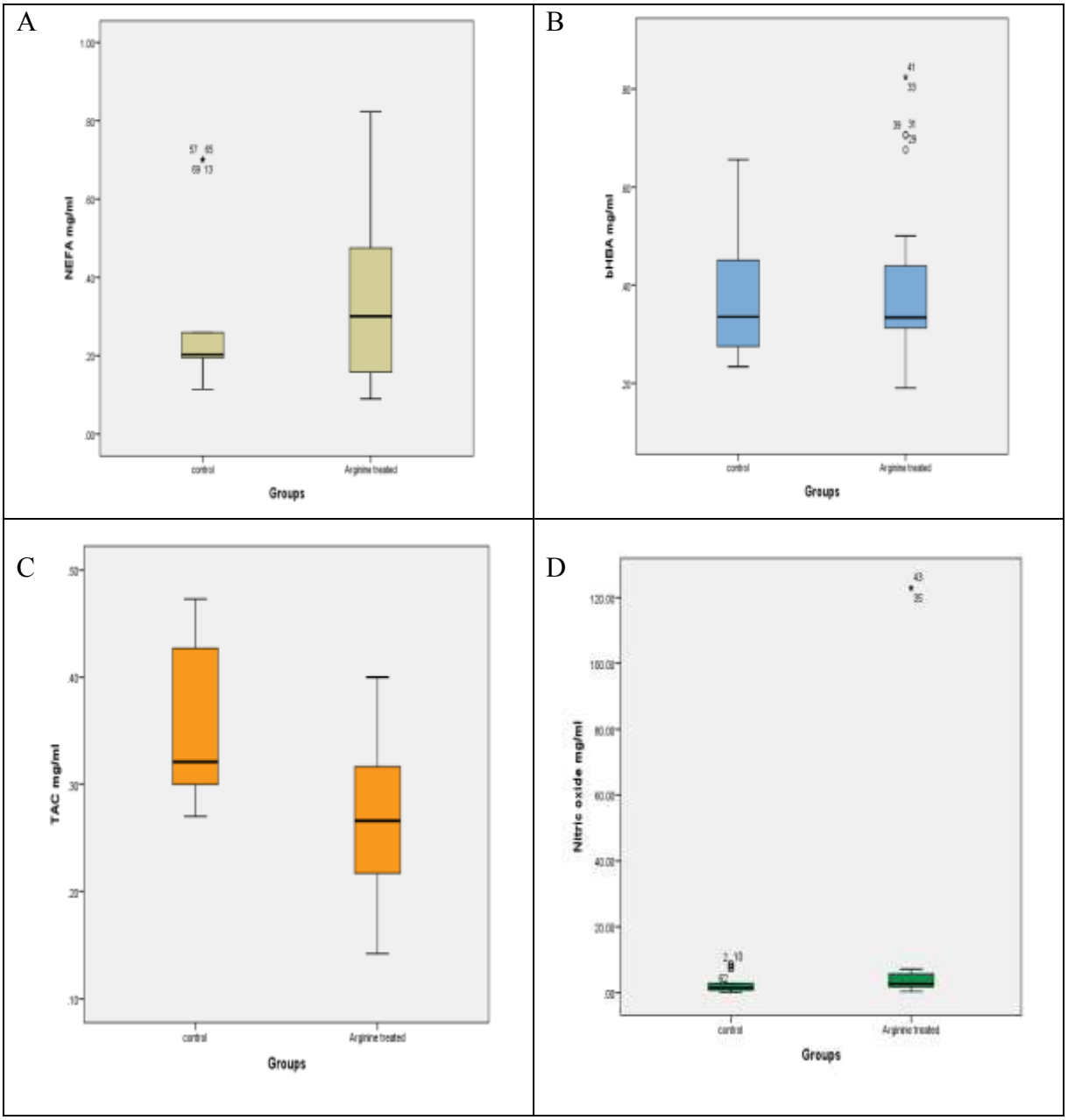

Figure 1. Comparison of NEFA (A), bHBA (B),TAC (C), and Nitric Oxide (D) levels at the end of the study between the control and experimental (arginine treated) groups.

Discussion

The main hypothesis of the present study was that the use of arginine increases milk production while no significant increase was observed in our results.The lack of milk increase in the present article is consistent with the results of some research. Twelve days infusion of 25 g/day L-arginine in goats could not increase milk production and concentrations of bST in plasma(Gow et al, 1979).

The lack of milk increase in the present paper conflicts with the results of some studies. It is reported that daily injections of 0.1/kg body weight L-arginine into the jugular vein of 8 cows during a 4 to 5-min period for about 7 d just before parturition caused plasma concentrations of prepartum bST, prolactin, and insulin, and postpartum milk production to increase (Chew et al, 1984).On the other hand, infusion of Arg via the jugular vein from 20 days after calving for one week in 6 Holstein cows had a positive effect on the synthesis of milk protein (Ding et al, 2019). One of the possible reasons for not increasing milk with Arginine consumption in the present study could be the low amount of medication used.We used about 19.2 g per injection in Holstein cows with about 600 kg, but Chew (1984) used about 60 grams per day of arginine and observed an increase in milk production. Nevertheless, Vicini et al, (1988) could not increase milk production of cows by infusion or intravenous injection of L-arginine. They concluded that the lack of changes in milk production and milk composition suggest that acute increases in somatotropin with concomitant increases in insulin are not sufficient to stimulate the synthesis of milk and milk components by cows during established lactation.In most trials, the intravenous injection of arginine was for a short time, usually about 5 min, and produced a brief increase in plasma hormone concentrations (Hertelendy et al, 1968; Chew et al, 1984; Hertelendy et al, 1969; Hertelendy et al, 1970; McAtee, 1971; McAtee and Trenkle, 1971; Davis, 1972). Repeated injections or perfusion of the drug into the serum may have better results in milk production. Interestingly, similar to the present study, Vicini et al, (1988) used L-arginine after the peak of milk lactation; similarly, they did not observe an increase in milk production. Increasing the duration of arginine injection increases the possibility of increasing milk production. The reason for our limited use of arginine was the fear of its potential dangers such as insulin resistance, ketosis and increased BHAB and NEFA.

Another hypothesis of the study was that if the injection of arginine increases milk production, it may reduce the concentration of fat and protein in milk, but none of the desired changes were observed.

The confounding variables were compared because their differences could affect milk or fat and milk protein production apart from the arginine effect.The similarity of confounding variables such as age, parity, SCC, and the intervals between parturition to the beginning of the study between the two groups showed that the amount of milk or fat and milk protein production is not affected and only the independent variable (use of arginine) can affect them.

Since arginine causes an increase in NO nitric oxide (Moncada and Higgs, 1993; Mepham, 1982), changes in arginine could be due to an increase of NO serum concentration; so, it was evaluated but no change was observed between groups.

On the other hand, increasing the absorption, excretion, and catabolism of fatty acids may also provide a possibility of altering the release of reactive oxygen species (ROS) (Seifert et al. 2010), so serum concentration of TAC was used to measure the release of reactive oxygen species (ROS). In our findings, TAC decreased, which could be a sign of the consumption of effective factors in active oxygen control. According to the findings

of Zheng et al, (2013), the use of 1.5% L-arginine supplementation caused oxidative stress in weaned piglets. The findings of Zheng et al, (2018) on low-birth-weight piglets showed that the use of 1% L-arginine oral supplement in a route separate from the nitric oxide pathway increased the antioxidant capacity.

It was concluded, from this study, that the injection of arginine in Holstein cows did not change fat, protein, and milk production.

Acknowledgments

The authors would like to thank the manager and thestaff of Saffary dairy farms for their supports in the implementation ofthe present research.

Conflict of interest

The authors declare that they have no conflict ofinterest.

Funding

The research was funded by the first author, Dr. Ali Tirgary because he used its results to produce a DVM thesis.

References

Azarbayejani, R., & Mohammadsadegh, M. (2021). Glucose, insulin, and cortisol concentrations and glucose tolerance test in Holstein cows with inactive ovaries. *Tropical Animal Health and Production*, *53*(1), 41. doi.org/10.1007/s11250-020-02448-7

Burton, J. L., McBride, B. W., Block, E., Glimm, D. R., & Kennelly, J. J. (1994). A review of bovine growth hormone. *Canadian Journal of Animal Science*, *74*(2), 167-201. doi: 10.4141/cjas94-027.

Chiofalo, V., Baldi, A., Savoini, G., Polidori, F., Dell'Orto, V., & Politis, I. (1999). Response of dairy ewes in late lactation to recombinant bovine somatotropin. *Small Ruminant Research*, *34*(2), 119-125. doi: 10.1016/S0921-4488(99)00061-9.

Chew, B. P., Eisenman, J. R., & 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.

Clark, J. H. (1975). Lactational responses to postruminal administration of proteins and amino acids. *Journal of Dairy Science*, *58*(8), 1178-1197.

Davis, S. L. (1972). Plasma levels of prolactin, growth hormone, and insulin in sheep following the infusion of arginine, leucine and phenylalanine. *Endocrinology*, *91*(2), 549-555.

Ding, L., Shen, Y., Wang, Y., Zhou, G., Zhang, X., Wang, M., ... & Zhang, J. (2019). Jugular arginine supplementation increases lactation performance and nitrogen utilization efficiency in lactating dairy cows. *Journal of animal science and biotechnology*, *10*(1), 1-10. https://doi.org/10.1186/s40104-018-0311-8

Disenhaus, C., Jammes, H., Hervieu, J., Ternois, F., & Sauvant, D. (1995). Effects of recombinant bovine somatotropin on goat milk yield, composition and plasma metabolites. *Small Ruminant Research*, *15*(2), 139-148.. doi:10.1016/0921-4488(94)00019-4

Gow, C. B., Ranawana, S. S. E., Kellaway, R. C., & McDowell, G. H. (1979). Responses to post-ruminal infusions of casein and arginine, and to dietary protein supplements in lactating goats. *British Journal of Nutrition*, *41*(2), 371-382.

Hayashi, A. A., Nones, K., Roy, N. C., McNabb, W. C., Mackenzie, D. S., Pacheco, D., & McCoard, S. (2009). Initiation and elongation steps of mRNA translation are involved in the increase in milk protein yield caused by growth hormone administration during lactation. *Journal of Dairy Science*, *92*(5), 1889-1899.doi: 10.3168/jds.2008-1334 PMID: 19389947.

Hertelendy, F., Machlin, L., & Kipnis, D. M. (1969). Further studies on the regulation of insulin and growth hormone secretion in the sheep. *Endocrinology*, *84*(2), 192-199.

Hertelendy, F., Machlin, L. J., Takahashi, Y., & Kipnis, D. M. (1968). Insulin release from sheep pancreas in vitro. *Journal of Endocrinology*, *41*(4), 605-606.

Hertelendy, F., Takahashi, K., Machlin, L. J., & Kipnis, D. M. (1970). Growth hormone and insulin secretory responses to arginine in the sheep, pig, and cow. *General and comparative endocrinology*, *14*(1), 72-77.

Lassala, A., Bazer, F. W., Cudd, T. A., Li, P., Li, X., Satterfield, M. C., ... & Wu, G. (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.

McATEE, J. W., & TRENKLE, A. (1971). Effects of feeding, fasting, glucose or arginine on plasma prolactin levels in the bovine. *Endocrinology*, *89*(3), 730-734.

McAtee, J. W., and A. Trenkle. 1971. Metabolic regulation of plasma insulin levels in cattle. J. Anim. Sci. 33:438.

Mepham, T. B. (1982). Amino acid utilization by lactating mammary gland. *Journal of dairy science*, *65*(2), 287-298. doi.org/10.3168/jds.S0022-0302(82)82191-7

Moncada, S., & Higgs, A. (1993). The L-arginine-nitric oxide pathway. *New England journal of medicine*, *329*(27), 2002-2012.

National Research council (2001). Nutritional Requirement of Dairy cows.Seventh Revised Edition.ISBN: 978-0-309-06997-7.doi.org/10.17226/9825.

Oliveira, L. H., Nascimento, A. B., Monteiro Jr, P. L. J., Guardieiro, M. M., Wiltbank, M. C., & Sartori, R. (2016). Development of insulin resistance in dairy cows by 150 days of lactation does not alter oocyte quality in smaller follicles. *Journal of dairy science*, *99*(11), 9174-9183.doi.org/10.3168/jds.2015-10547.

Sallam, S. M. A., Nasser, M. E. A., & Yousef, M. I. (2005). Effect of recombinant bovine somatotropin on sheep milk production, composition and some hemato-biochemical components. *Small Ruminant Research*, *56*(1-3), 165-171.

Seifert, E. L., Estey, C., Xuan, J. Y., & Harper, M. E. (2010). Electron transport chain-dependent and-independent mechanisms of mitochondrial H2O2 emission during long-chain fatty acid oxidation. *Journal of Biological Chemistry*, *285*(8), 5748-5758..DOI 10.1074/jbc.M109.026203

Squires, E. J. (2003). Endocrine manipulation of reproduction. In *Applied animal endocrinology* (pp. 154-191). Wallingford UK: CABI Publishing.

Vicini, J. L., Clark, J. H., Hurley, W. L., & Bahr, J. M. (1988). Effects of abomasal or intravenous administration of arginine on milk production, milk composition, and concentrations of somatotropin and insulin in plasma of dairy cows. *Journal of dairy science*, *71*(3), 658-665.

Wang, M., Xu, B., Wang, H., Bu, D., Wang, J., & Loor, J. J. (2014). Effects of arginine concentration on the in vitro expression of casein and mTOR pathway related genes in mammary epithelial cells from dairy cattle. *PLoS One*, *9*(5), e95985..

Zheng, P., Song, Y., Tian, Y., Zhang, H., Yu, B., He, J., ... & Chen, D. (2018). Dietary arginine supplementation affects intestinal function by enhancing antioxidant capacity of a nitric oxide–independent pathway in low-birth-weight piglets. *The Journal of nutrition*, *148*(11), 1751-1759. doi:https://doi.org/10.1093/jn/nxy198.

Zheng, P., Yu, B., He, J., Tian, G., Luo, Y., Mao, X., ... & Chen, D. (2013). Protective effects of dietary arginine supplementation against oxidative stress in weaned piglets. *British journal of nutrition*, *109*(12), 2253-2260.

Received: 27.10.2022
Accepted: 30.11.2022

DOI: 10.22055/IVJ.2022.360735.2520
DOR: 20.1001.1.17356873.1402.19.1.9.4

بررسی سطح چربی، پروتئین و تولید شیر در گاوهای شیری هلشتاین تیمار شده با آرجنین

علی تیرگری[۱]، مجید محمدصادق[۲*]، مرتضی گرجی‌دوز[۳]، علی افشار بهرآباد[۴] و نیما فرزانه[۵]

[۱] دانش‌آموخته دکترای عمومی، دانشکده دامپزشکی، واحد گرمسار، دانشگاه آزاد اسلامی، گرمسار، ایران
[۲] دانشیار، گروه علوم درمانگاهی، دانشکده دامپزشکی، واحد گرمسار، دانشگاه آزاد اسلامی، گرمسار، ایران
[۳] استادیار، گروه علوم درمانگاهی، دانشکده دامپزشکی، واحد گرمسار، دانشگاه آزاد اسلامی، گرمسار، ایران
[۴] دانش آموخته دکترای تخصصی، گروه مامایی و بیماری‌های تولید مثل، دانشکده دامپزشکی، دانشگاه فردوسی مشهد، مشهد، ایران
[۵] استاد، گروه علوم درمانگاهی، دانشکده دامپزشکی، دانشگاه فردوسی مشهد، مشهد، ایران

تاریخ دریافت: ۱۴۰۱/۸/۵ تاریخ پذیرش: ۱۴۰۱/۹/۹

چکیده

در این مطالعه ۷۳ رأس گاو هلشتاین غیر آبستن سالم شیرده و از نظر بالینی بدون هیچ علامت بالینی و با میانگین تعداد زایش ۱/۴±۳/۲ روزهای شیردهی ابتدای مطالعه ۲۰±۱۱۰ روز، وضعیت بدنی ۰/۳±۳/۲ و میزان تولید شیر ۱۰±۴۸ کیلوگرم انتخاب شدند و به طور تصادفی در دو گروه تیمار و کنترل قرار گرفتند. در گروه درمان (n=36) آرجنین (۱۵۵ میکرومول بر کیلوگرم وزن بدن) هر ۸ ساعت یک بار در روز به مدت ۶ روز و در گروه شاهد (n=37) محلول نمکی (۰/۱۱ میلی‌لیتر بر کیلوگرم وزن بدن) هر ۸ ساعت یک بار در روز) به مدت ۶ روز تزریق شد. نتایج نشان داد که تولید شیر (۳۸/۵ و ۳۶ کیلوگرم، P=۰/۳)، چربی (۳/۷ و ۳/۸ درصد، P=۰/۸) و پروتئین (۳/۱ و ۳/۱ درصد، P=۰/۵) به ترتیب در گروه تیمار و شاهد تفاوتی نشان ندادند. نتایج به دست آمده نشان می‌دهد که در شرایط این مطالعه، استفاده از آرجنین پس از پیک تولید شیر نمی‌تواند باعث افزایش چربی، پروتئین و سطح تولید شیر شود.

کلمات کلیدی: آرجنین، آمینواسید، تولید شیرگاو هلشتاین، پروتئین و چربی شیر

* **نویسنده مسئول: مجید محمدصادق**، دانشیار، گروه علوم درمانگاهی، دانشکد دامپزشکی، واحد گرمسار، دانشگاه آزاد اسلامی، گرمسار، ایران
E-mail: Majid.Mohammadsadegh@iau.ac.ir