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Significance of rumen protected L-arginine – supplementation on certain blood parameters, mammary gland functions and growth rate of newly born lambs

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Abstract

The objective of this study was to investigate the effect of rumen-protected L-arginine (ARG) on the udder of ewes and growth rate of newly born lambs. At 15±3 days postpartum, ten clinically healthy, 3-4 years ewes with body weight 49.11±4.03 Kg were divided randomly into 2 equal groups Group 1: served as control, without any treatment. Group 2: treated with Rumen protected L-Arginine 20 Mg/Kg body weight, for 30 days. The animals were examined with ultrasound, and blood samples were taken at the 10th, 20th and 30th days of ARG supplementations begin, and the lambs were weighted in the same times. The results of this study revealed that significant differences (P<0.05), between ARG treatment and control one, in some udder measurement's. The glucose concentration after 10, 20, and 30 days of treatment showed significant differences (P<0.01, and 0.05), where the ARG treatment had higher glucose concentration than control. Also, there were significant differences (P<0.05), between ARG treatment and control group, in the urea and AST concentrations. There was an increase in the lamb's body weight at 10th, 20th and 30th Day of ARG treatment. The ARG supplementation appeared to have significant effects on postpartum udder measurements as well as the growth rate of the newly born lambs.

Keywords: L-arginine, mammary gland, ultrasound, biochemical blood, newly born lamb.

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1. Introduction

Arginine is one of the twenty common amino acid (AA) used to synthesize all proteins, including milk protein, (NRC, 2001). However, rumen protected arginine, plays multiple roles in the body, including metabolism, immune function, and hormone secretion, (Wu, 2009). The metabolic end products of Arg-Orn and Arg-NO are polyamines (putrescine, spermidine, and spermine) and nitric Oxide (NO), respectively. Some studies have reported that putrescine had positive effects on the proliferation of cells, addition putrescine in growth medium promoted the synthesis of protein in porcine trophectoderm cells. Furthermore, spermidine has also been reported to be associated with milk protein synthesis in mammary gland (Wu *et al.,* 2009). Injection of Arg increased plasma concentrations of somatotropin, insulin, prolactin, and placental lactogen (Vicini *et al.,* 1988). In addition that ARG supplementation may reduce whole-body amino acid degradation and, increase, urea production as well as the endogenous synthesis of glutamine from branched-chain amino acids and ammonia, (Mateo *et al.,* 2007). The arginine plays multiple roles in the body metabolism (Wu, 2009), and that the response in mammary metabolism in terms of both energy and protein may vary with amino acid supply (Lemosquet *et al.,* 2010), and depressed milk yield in adult ewes, indicated that thyroid dysfunction (Sipos *et al.,* 2004). The objective of this study was to Investigation the effect of rumen-protected L-arginine Supplementation on the ewe's udder by ultrasonography during the post-partum and growth rate of the newly born lambs.

2. Materials and methods

2.1 Animals and Managements

The experiment was conducted during the period from September 2017 to October 2017 proceeding in accordance with the Ethics Committee on Animal Experimentation of Al-Azhar University faculty of agriculture. Ten pluriparous ewes with a body weight of 40-50 kg were included. The animals belong to the farm of Al-Azhar University, Faculty of Agriculture, Assuit, Egypt. The ewes were housed in sheds during the experimental periods. The animals were clinically healthy, free from reproductive disorders and fed on daily farm ration, water, and a mineral supplement were available adlibitum. After 15±3 days of parturition, the ewes divided into 2 equal groups. Group 1: served as control, without any treatment. Group 2: treated with Rumen protected L-Arginine 20 Mg/Kg body weight (l-arginine pure, 25 gm, C6H14N4O2, Edappally, India, imported by El-goumhouria Co, Cairo, Egypt), for 30 days (Figure 1).

2.2 Bypass L-arginine pellets

L-arginine in the laboratory was modified to avoid ruminal degradation; this modification was composed of two different layers (Julio *et al.,* 2015). The first layer consisted of L-arginine and barium sulfate (El-Goumhouria Co,

Cairo, Egypt) nonfunctional polymer, L-Arginine was added to the cellulose acetate phthalate by 1: 1 and then mixed well, after then, add the barium sulfate on the previous mixture. The incorporation of barium sulfate served the purpose of providing density to the pellets to prevent them from being damaged by rumination. The outer layer consisted of a functional polymer Eudragit® S100; (imported by Memphis pharm and chemical Co, Cairo, Egypt), will be prepared by Ether solution, with the capacity to dissolve at pH above 7.2, which thus enabled them to release of L-arginine in the intestine. The ratio between the L-Arginine and the polymer was 4:1 to form the billet. The amount of L-Arginine in the pellets was evaluated according to the method of Moore and Stein (1954).

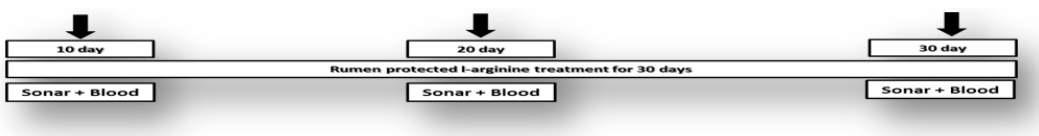

Figure (1): Protocol of ewe's groups during lactation.

2.3 Ultrasonography for the udder

Ultrasonographic imaging of the udder and teats was carried out on ewes in the standing position, 2 hours after sucking by lambs, with a real-time, B-mode, diagnostic scanner equipped with a linear array multi-frequency 5/7.5 MHz transducer (Hitachi, EUB-405B, Japan), Images were frozen on the monitor of the ultrasound scanner and dimensions of these organs were measured at their maximum with integrated caliper in ultrasound device. The examination method was "direct method" ultrasonography; the probe was placed on the skin after using of a contact gel. During the scan, the transducer was positioned along the length and width of the mammary gland, designated, respectively, as sagittal and horizontal positions. Measurements the following were taken: udder width (UW), udder depth (UD), udder cistern (UC), diameter of teat cistern (DTC), teat canal (TC), and teat cross section (TCS), in the right and left halfs of the udder, (Figure, 2). Were measurements in ultrasonography scans as described by Enda and Dinç (1999), were recorded in three-times, after 10^{th}, 20^{th} and 30^{th} Day, from starting the feed with rumen-protected L-arginine.

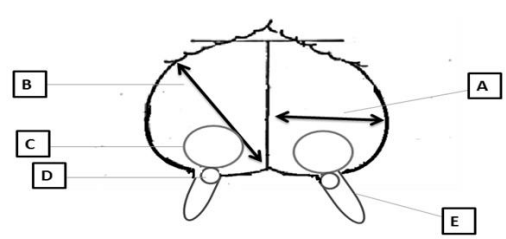

Figure (2): Diagram illustration for the udder measurements of the ewe by ultrasonography. A: Udder Width, B: Udder Depth, C: Udder Cistern, D: Diameter of Teat Cistern and E: Teat Canal.

3. Results and Discussion

3.1 Ultrasonic appearance of the udder in ewes administrated rumen-protected L-arginine

After 10 days of starting the administration of rumen-protected L-arginine to ewes there were no statistical differences in all measurements including; RUC, RDTC and TCS between the right and left half's of the udder. However, highly significant differences (P<0.01), between ARG treatment and control one, in RUD, LUD and LDTC, Also, the ARG treatment has significant differences (P<0.05), then control one, on RUW, right teat canal (RTC), LUW, LUC, and (LTC) left teat canal (Table 1). A work on arginine in ruminant mammary gland has been undertaken in 2011; their results showed that milk protein yield was increased by infusion of Arginine compared with the control, and there were Studies reported that the ARG might have plays multiple roles in the body, including metabolism, immune function, and hormones secretion (Mabjeesh *et al.,* 2002). Injection of ARG increased plasma concentrations of somatotropin, insulin, prolactin, and placental lactogenic (Vicini *et al.*, 1988).

Table (1): Effect of the rumen protected L-arginine on the right and left ewe's udder.

		The right udder					
	Treatment	RUW[1]	RUD[2]	RUC[3]	RDTC[4]	RTC[5]	RTCS[6]
After 10 days of starting ARG	control	2.44±0.11	2.43±0.14	0.75±0.03	0.54±0.01	0.38±0.03	1.27±0.16
	ARG	3.80±0.41	3.73±0.34	1.03±0.16	0.66±0.07	0.82±0.13	1.43±0.11
	Sig	0.05	0.01	NS	NS	0.05	NS
After 20 days of starting ARG	control	3.05±0.35	2.87±0.28	0.88±0.05	0.51±0.02	0.44±0.02	0.97±0.09
	ARG	4.13±0.19	4.29±0.28	1.37±0.16	0.70±0.07	0.62±0.08	1.44±0.07
	Sig	0.05	0.01	0.05	0.05	0.05	0.01
After 30 days of starting ARG	control	3.32±0.18	3.36±0.19	0.91±0.07	0.44±0.03	0.70±0.10	0.75±0.18
	ARG	4.44±0.16	4.36±0.14	1.49±0.12	0.89±0.09	0.73±0.13	1.32±0.04
	Sig	0.01	0.01	0.01	0.01	NS	0.05
		The left udder					
	Treatment	LUW[7]	LUW[7]	LUW[7]	LUW[7]	LUW[7]	LUW[7]
After 10 days of starting ARG	control	2.61±0.33	2.61±0.33	2.61±0.33	2.61±0.33	2.61±0.33	2.61±0.33
	ARG	3.65±0.23	3.65±0.23	3.65±0.23	3.65±0.23	3.65±0.23	3.65±0.23
	Sig	0.05	0.05	0.05	0.05	0.05	0.05
After 20 days of starting ARG	control	2.86±0.31	2.86±0.31	2.86±0.31	2.86±0.31	2.86±0.31	2.86±0.31
	ARG	4.25±0.09	4.25±0.09	4.25±0.09	4.25±0.09	4.25±0.09	4.25±0.09
	Sig	0.01	0.01	0.01	0.01	0.01	0.01
After 30 days of starting ARG	control	2.86±0.31	2.86±0.31	2.86±0.31	2.86±0.31	2.86±0.31	2.86±0.31
	ARG	4.02±0.13	4.02±0.13	4.02±0.13	4.02±0.13	4.02±0.13	4.02±0.13
	Sig	0.05	0.05	0.05	0.05	0.05	0.05

Different superscript letters indicates significance within the same column (p<0.05).

1. RUW: Right Udder Width
2. RUD: Right Udder Depth
3. RUC: Right Udder Cistern
4. RDTC: Right Diameter of Teat Cistern
5. RTC: Right Teat Canal
6. RTCS: Right Teat Cross Section
7. LUW: Left Udder Width
8. LUD: Left Udder Depth
9. LUC: Left Udder Cistern
10. LDTC: Left Diameter of Teat Cistern
11. LTC: Left Teat Canal
12. LTCS: Left Teat Cross Section

Perhaps the increase in the growth of mammary cells in this study is a reference to these reasons. To our knowledge, there aren't ultrasonic studies on the udder measurements in ewes fed L-arginine. After the 20th Day of rumen-protected L-

arginine feeding, there are significant (P<0.001) differences between ARG group and control one in RUD, RTCS, LUW, LUD, and LTC). Also, there were significant differences (P<0.05) between ARG and control group in RUW, RDTC, RTC, LDTC, and LTCS, as shown in table 1. Because the arginine plays a role in tissue function and enzyme activity, however, the nitric oxide (NO) this becomes a powerful vasodilator and biological regulator of tissues (Wu *et al.*, 2006; Arciszewski 2007). After 30 days of starting the feeding with rumen-protected L-arginine, there were no statistical differences between the right and left side measurements. However, highly significant differences (P<0.01), between ARG treatment and control one in RUW, RUD, RUC, RDTC, LUC and LTC, as shown in table 15 and figure 20. Also, there were significant differences (P<0.005), between ARG treatment and control one, on, RTCS, LUW, and LDTC. The metabolic end products of (Arg-Ornithine and Arg-Nitric oxide) are polyamines (putrescine, spermidine, and spermine) and NO, respectively. However, some studies have reported that putrescine had positive effects on the proliferation of cells, addition putrescine in growth medium promoted the synthesis of protein in porcine trophectoderm cells. Furthermore, spermidine has also been reported to be associated with milk protein synthesis in mammary gland (Wu *et al.*, 2009). However, elevated NO levels stimulate mitochondrial biogenesis by up-regulating expression of peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1α) (Nisoli *et al.,* 2005).

3.2 Glucose, urea, Aspartate aminotransferase (AST) and Alanine aminotransferase (AlT) concentrations in lactation ewes during Arginine treatment

The glucose concentration in the three time (after 10, 20, and 30 days) of starting the feed rumen-protected L arginine, have significant differences (P<0.01, and 0.05) in the ARG treatment higher than control. Also, there were significant differences (P<0.05), between ARG treatment and control group, in the urea and AST concentrations after 20 days of treatment (55.53±2.06 *vs*. 49.62±1.43), and (43.20±0.58 *vs*. 41.00±0.70), respectively, and ALT concentration after 30 days of treatment (34.20±1.88 vs. 25.46±0.04), as shown in Table (2). The effects of arginine on glucagon secretion are employing the perfused pancreas technique which indicates that the pancreatic alpha cell is quite responsive to arginine (Pagliara *et al.*, 1973). Increased levels of the glucose concentration in ewes during treatment with arginine may be indicated of increased activity of mammary cells as shown in tables 1. In addition that ARG supplementation may reduce whole-body amino acid degradation and, increase, urea production as well as the endogenous synthesis of glutamine from branched-chain amino acids and ammonia, (Mateo *et al.,* 2007). There were no statistical differences in the urea, and AST concentrations after 10, 30 days of treatment, ALT concentration after 10, 20 days of treatment, between groups, although there was a clear increase in the ARG group from the control group, this increase was not significant, (Table 2).

This result agreement with Tainturer *et al.* (1984) found that AST activity in dairy cows changes irregularly and occasionally during pregnancy and lactation, but that these changes were not statistically significant. However Zvonko *et al.* (2005), reported that low in the AST concentration (during the lactation period) after 46 days than 91 days of lactation (57.79±16.49 *vs.* 44791±6.93), and there were significant in the ALT concentration in the same times (8.91±2.23 *vs.* 20.08±3.74). In addition the Arginine is known to play a role in tissue function and enzyme activity (Wu *et al.*, 2006). However, ALT levels in the blood stream are playing a catalytic role in the synthesis of amino acid (Gbolabo *et al.*, 2017). Therefore, this may be the reason for the high level of ALT concentration.

Table (2): Effect of the rumen protected L arginine on glucose, urea, AST and ALT concentrations in ewes during lactation.

	Treatment	After 10 Days	After 20 days	After 30 days
Glucose	Control	70.78±2.27	76.58±5.01	80.04±2.62
	ARG	84.64±4.98	97.62±1.79	94.98±1.90
	P	0.05	0.01	0.01
Urea	Control	52.18±1.77	49.62±1.43	45.98±1.12
	ARG	50.12±2.89	55.53±2.06	48.27±1.75
	P	NS	0.05	NS
AST	Control	37.00±1.73	41.00±0.70	42.25±1.65
	ARG	37.00±0.44	43.20±0.58	45.60±1.56
	P	NS	0.05	NS
ALT	Control	26.80±1.59	27.06±1.64	25.46±0.04
	ARG	28.60±2.97	31.20±1.06	34.20±1.88
	P	NS	NS	0.05

Different superscript letters indicates significance within the same column ($p<0.05$).

3.3 The levels of serum thyroid hormones T3 and T4 in ewes during Arginine treatment

The T3 concentration in the third time (after 30 days) of starting the feed rumen-protected L arginine, have significant differences ($P<0.05$) in the ARG treatment higher than control, (0.20±0.03vs. 0.08±0.01), respectively. While T4 concentration (after 10, 20 and 30 days of starting treatment), had significant differences ($P<0.05$), in the ARG treatment higher than control. Serum levels of T3, T4 are used as reliable indicators of the thyroid function in animals (Kelly, 2000). In addition, the thyroid gland plays an important role in energy usage, synthesis of RNA protein, consumption of oxygen by cells, overall body metabolism, growth processes and neurological development (Vanderpump and Tunbridge, 2008). The Arginine treatment had a positive effects on glucose concentration (table 2), In addition, the arginine plays multiple roles in the body metabolism (Wu, 2009), and that the response in mammary metabolism in terms of both energy and protein may vary with amino acid supply (Lemosquet *et al.*, 2010). And depressed milk yield in adult ewes, indicated that thyroid dysfunction (Sipos *et al.*, 2004).

Table (3): Effect of the rumen protected L-arginine on the levels of serum thyroid hormones T3 and T4 in ewes.

	Treatment	After 10 Days	After 20 days	After 30 Days
T3	Control	0.06±0.01	0.14±0.04	0.08±0.01
	ARG	0.12±0.03	0.17±0.02	0.20±0.03
	sig	NS	NS	0.05
T4	control	0.18±0.02	0.19±0.01	0.22±0.06
	ARG	0.28±0.02	0.36±0.04	0.38±0.03
	sig	0.05	0.05	0.05

Different superscript letters indicates significance within the same column ($p<0.05$).

3.4 3.4 Effect of the rumen protected L arginine, on the lamb's body weight, during dam's treatment

There were significant differences ($P<0.05$), between ARG treatment and control one, in the lamb's body weight at the day of treatment (after 7 days of birth). Also, there was an increase in the lamb's body weight during dam's treatment by rumen-protected L arginine, (after 10, 20, and 30 days of treated), between ARG and control groups (Table 4). There were improved in the udder measurements by arginine treatment (udder width, depth, and cistern, diameter of teat cistern and teat canal), as showed in table 13, 14 and 15 during lactating period in ewes. Moreover, the arginine has positive effects on the proliferation of cells, synthesis of protein in porcine trophectoderm cells, and milk protein synthesis in mammary gland (Chen *et al.*, 2015; Kong *et al.*, 2014; Wu *et al.*, 2009).

Table (4): Effect of the rumen protected L-arginine, on the lamb's body weight, during dam's treatment.

Treatment	Birth Weight	0 day (after 7 d)	After 10 day	After 20 day	After 30 day
Control	3.01±0.26	5.22±0.24	7.25±0.53	10.10±0.24	13.47±0.16
Arg	2.88±0.06	4.57±0.13	9.10±0.31	11.78±0.67	15.39±0.63
Sig	NS	0.05	0.05	0.05	0.05

Different superscript letters indicates significance within the same column ($p<0.05$).

These functions could improve the quality of the milk intake for the lambs during the lactation period, thus improving the weight of the body during feeding with arginine**.**

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