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Nutrients Requirements in Pregnant Ewes is L-Arginine or Glucose Dependence Required for Fetal Growth, Survival and Maternal Progesterone During Late Pregnancy in Ewes

Moustafa M Zeitoun*

Department of Animal Production and Breeding, College of Agriculture and Veterinary Medicine, Qassim University, Saudi Arabia

***Corresponding author:** Moustafa M Zeitoun, Department of Animal Production and Breeding, College of Agriculture and Veterinary Medicine, P.O. Box 6622, Qassim University 51452, Saudi Arabia. Tel:+966559940331; Fax: +966163801360; Email:mmzeitoun@yahoo.com

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Abstract

This study aimed at investigating effects of drenching low and high dosage of L-arginine alone, combined with propylene glycol or propylene glycol alone during late ewe's gestation on the litter traits and maternal progesterone (P4). Thirty four adult Najdi ewes were allotted into six groups (G). G1 (C, control) ewes orally given physiological saline daily, G2 (LA) ewes given 37.5 mg L-arginine/kg/day, G3 (HA) ewes given 75 mg l-arginine/kg/day, G4 (P) ewes given propylene glycol, G5 (LAP) ewes given a mixture of propylene glycol and LA and G6 (HAP) ewes given a mixture of propylene glycol and HA. Blood samples for P4 were collected once a week till parturition. Litter size, litter weight, neonatal birth weight and viability were determined. Mean lamb birth weight was 3.75, 5.44, 5.72, 5.75, 7.33 and 5.6 kg in C, LA, HA, P, LAP and HAP, respectively. The highest P4 level ($P<0.05$) with typical profile was found in LAP ewes. Alternatively, mean P4 didn't change ($P>0.05$) in other treatmentsthan control. In conclusion, administration of a combination of L-arginine (37.5 mg/kg/d) with propylene glycol during late sheep pregnancy not only enhanced neonatal weight and survival but it also enhanced maternal corpus luteum function

Keywords: Fetal Growth; L- Arginine; Progesterone; Propylene Glycol; Ovine Pregnancy

Introduction

Maternal nutritional status in animal species is one of the factors implicated in programming nutrient partitioning and ultimately growth, development and function of the major fetal organ systems[1-4]. In most animal species in general and sheep in specific almost 70-80% of fetal growth occurs during the last trimester of pregnancy [5]. Less feed resources in desert areas around the world represents a challenge for successful animal production. Recently, the trend of new research in animal feeding is to compensate for the lack of feedstuffs by some efficient additives. The linear growth of sheep fetuses in late pregnancy makes it more difficult for the ewe to meet various maternal and fetal requirements. Recent studies revealed the importance of the amino acid L-Arginine during pregnancy in ewes [6]. Moreover, the use of extra energy sources in the diet of late pregnant ewes has been a focus especially in twin-bearing ewes[7].

Propylene Glycol (PG) is a 3-carbon compound derived from propylene which has applications in both industry and medicine. Propylene glycol provides the ruminants with glucose and used as a drench in the treatment of ketosis in

animals[8]. Initial observation on using PG during late gestation revealed an increase of maternal blood glucose and heavier neonatal birth weight[9]. Administration of PG during per parturient period in lactating dairy cows enhanced some hematological parameters after parturition[10-13] have shown the gluconeogenic property of PG, as reflected by an elevation in blood glucose when it was fed to dairy cows. The elevation in blood glucose concentration when PG was fed to dairy cows has also been shown to lead to a concomitant increase in blood insulin concentration [8,11,13,14]. Up to date none of the studies has looked on the combinations of a source of carbohydrate and a source of protein/ amino acids on the fetal growth, development and survival concomitantly with maternal health. Whether sheep fetal growth at late gestation requires a source of energy (i.e. PG) or a source of protein (i.e. L-Arginine) is the focus of the present study.

Materials and Methods

Animals, location and management

This study was carried out during March-July 2014. Thirty-four late pregnant Najdiewes were housed in semi-shaded pens located in the Qassim University experimental farm. Animals were fed on barley (300g/hd/day) and alfalfa hay (*ad libitum*), offered balanced mineral licks and free access to clean tap water. Due to the application of regular immunization schedule, animals were free of parasites and diseases. Permission was obtained from the animal rights and ethics of use unit of the Scientific Research Deanship of Qassim University.

Experimental design

Thirty-four Najdiewes were randomized into 6 treatments and were orally administered the designed solution for 56 days starting at day 80 of gestation as follow;

- G1 (C; n=6) ewes served as control group in which every ewe was given daily oral 50 ml normal physiological saline (0.9% NaCl) daily.
- G2 (LA; n=6) ewes were given daily oral administration (50 ml) of a solution containing low dosage of 37.5 mg L-Arginine/kg B.W.
- G3 (HA; n=6) ewes were given daily oral administration (50 ml) of a solution containing high dosage of 75 mg L-Arginine/kg B.W.
- G4 (P; n=6) ewes were given daily oral administration of 50 ml (equivalent to 50 g) propylene glycol (Ketosaid®, nor brook Lab, North Ireland).
- G5 (LAP; n=5) ewes were orally given daily mixture of 25 ml propylene glycol and 25 ml of the low Arginine solution.
- G6 (HAP; n=5) ewes were orally given daily mixture of 25 ml propylene glycol and 25 ml of the high Arginine solution.

Blood Sampling and Sera Harvesting

Jugular venipuncture blood samples were collected from 3 ewes randomly selected within each treatment. Sample collection started just before the application of the treatment (08:00 hr.) and continued every week until day of parturition. Blood samples were collected in plain Vacutainer® tubes and kept for two hours in refrigerator. Samples were centrifuged at 3000 rpm for 15 minutes at 5°C, sera were harvested in clean tubes and kept frozen (- 20°C) until progesterone was determined.

Progesterone Determination

Plasma progesterone was quantitated by the use of a commercial kit (HUMAN®, Germany) according to[15]**.** Samples were determined in duplicates. The sensitivity of progesterone ranges between 0.03 and 0.07 ng/ml. The intra- and inter-assay coefficient of variations for progesterone values were 3.7% and 5.1%, respectively.

Neonatal Parameters

At birth individual lambs and litter size were weighed and survival was determined. Also, gender of newborn was determined.

Final impact of the treatment (lamb crop)

This factor was proposed by the authorto find out the culminate impact of treatment on lamb crop. The following formula was proposed and applied within a treatment;

Treatment impact value = Lamb birth weight (kg) × Litter survival at birth (%)

Statistical analysis

Data of hormone concentrations were analyzed by the least square analysis of variances for repeated measuresby [16]**.**The one-way analysis of variances was applied using the following model.

$$Y_{ijk} = \mu + S_i + T_j + S_iT_j + e_{ijk}$$

Where:

- Y_{ijk}= an observation taken on the kth ewe;
- μ= overall mean;
- S_i= a fixed effect of the ith treatment (i = 6);
- T_j= a fixed effect of Jthweek of blood sample (j =10);
- S_iT_j = Treatment × Week;
- e_{ijk}= Random error assumed to be independent and normally distributed;
- With mean = 0 and variance = σ2e.

Differences among treatment means were tested by the Duncan Multiple Range Test [17].

Results

Off Spring Traits

As shown in (Table 1), mean lamb birth weight (7.33 kg) was highest (P<0.05) in ewes given a mixture of propylene and low dosage of L-Arginine (LAP) with a range of 6-10 kg. The second highest (P<0.05) birth weight was found in ewes given propylene alone (P; 5.75 kg). High Arginine (HA) alone resulted in higher lamb birth weight (5.72 kg) than when mixed with propylene (HAP; 5.6 kg). The lowest (P<0.05) lamb birth weight was found in ewes given low dose of Arginine alone (LA; 5.44 kg) even though it is still higher than control (C; 3.75 kg). Litter size has none due to treatment, however it varies among groups. The highest (P<0.05) lamb crop was obtained from ewes given propylene plus High Arginine (HAP) due to the highest litter size (i.e. 2).

Treatment	No. Ewes	Total lamb born	Live lamb born	% Lamb Survival	Total Litter Size Weight (Kg)/TRT	Live Litter Size Weight (Kg)/TRT	Litter Birth weight (kg)/ewe	Litter size	Mean Lamb Birth Weight (Kg)
Control (C)	6	8	6	75^{c}	30	22.5	5±0.25^{c}	1.33	3.75±0.20^{c} (3.5-4.5)§
Low Arg (LA)	6	8	7	87.5^{b}	43.5	38	7.25±0.35^{b}	1.33	5.44±0.32^{b} (4-7.5)
High Arg (HA)	6	9	9	100^{a}	51.5	51.5	8.58±0.52^{b}	1.5	5.72±0.40^{b} (5-6.5)
Propylene (P)	6	6	5	83.3^{b}	38	34.5	5.75±0.55^{c}	1	5.75±0.42^{b} (3.5-10)
LAP*	5	6	5	83.3^{b}	44	36.7	8.8±0.62^{b}	1.2	7.33±0.30^{a} (6-10)
HAP**	5	10	10	100^{a}	56	56	11.2±0.65^{a}	2	5.6±0.35^{b} (4-8)

*LAP = Propylene + Low Arginine; ** HAP = Propylene + High Arginine; §Range of lamb birth weight; Means in the same column with different superscripts significantly differ (P<0.05).

Table 1: Effect of oral administration of propylene glycol, L-Arginine or their mixture on Najdiewes litter traits (Mean±SEM).

Percentage of lamb survival at birth was 75, 87.5, 100, 83.3, 83.3 and 100% for C, LA, HA, P, LAP and HAP ewes, respectively. The highest (P<0.05) survival was found in the treatments applying the high dose of l-Arginine. In control ewes 4 ewes delivered singles and 2 ewes gave twins, likewise in LA group, 4 ewes gave singles and 2 ewes gave twins. However, in HA group, 3 ewes gave singles and 3 ewes gave twins. In ewes given propylene alone (P) all ewes gave singles, however in LAP group, 4 ewes gave singles and one ewe gave twins. In ewes given the mixture of propylene with the high Arginine (HAP), one ewe gave single, one ewe gave triplet and 3 ewes gave twins.

Maternal Progesterone

As illustrated in (Figure 1),theControl ewes (C) expressed typical progesterone profile with a mean of 7.84 ng/ml (Table 2) and 0.68 ng/ml at parturition. Comparable to what is found in control, ewes given the low dose of L-Arginine alone (LA) revealed similar P4 profile but with a higher (P<0.05) mean (11.08 ng/ml) and relatively similar P4 level at parturition (0.69 ng/ml). In ewes given the high dose of L-Arginine (HA) there obtained an irregular P4 profile with a mean similar to control (7.99 ng/ml) and P4 level of 0.55 ng/ml at parturition. A typical P4 profile was found in ewes given Propylene (P), however mean P4 during gestation didn't differ (5.21 ng/ml) than control. In LAP and HAP ewes there expressed typical P4 profiles with mean of 5.34 and 5.68 ng/ml, respectively. Although non-significant differences were found on P4 levels due to treatments (except LA), the existence of propylene either alone or in combination with L-Arginine tended to decrease P4 levels (Table 2; P>0.05). There found no statistical interaction (P>0.05) between treatment and week of sampling on P4 levels, whereas there obtained significant (P<0.05) changes of P4 due to sequence of week during pregnancy.

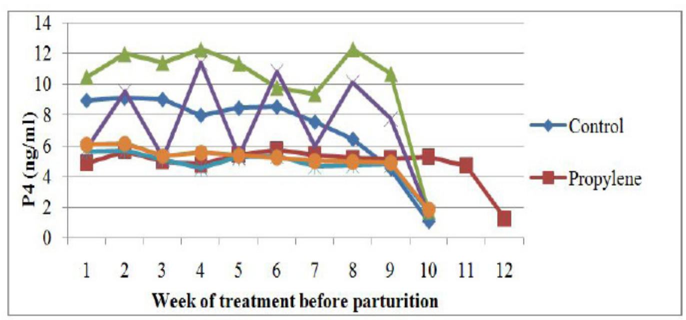

Figure 1: Progesterone profiles during gestation in Najdi ewes given propylene, Arginine or both.

Culminate impact of the treatment (lamb crop)

(Figure 2) exhibits the final outcome of the treatment on lamb crop.

All treatments resulted in higher impacts than control. The highest impact was obtained of the LAP ewes (6.11). However, similar impact was found among propylene (4.79) and low Arginine (4.76) ewes. High Arginine alone (HA; 5.72) surpassed the mixture of high Arginine with propylene (HAP; 5.6) in its final outcome. Overall, all treatments improved the lamb crop by 69-117% over control.

Treatment	Mean P4	P4 at Parturition
Control (C)	7.84±2.11[b]	0.56±0.32
Low Arg (LA)	11.08±1.01[a]	0.69±0.22
High Arg (HA)	7.99±1.87[b]	0.55±0.45
Propylene (P)	5.21±2.21[b]	0.76±0.42
LAP*	5.34±1.66[b]	0.77±0.32
HAP**	5.68±1.88[b]	0.80±0.45
***LAP** = Propylene + Low Arginine; ** HAP = Propylene + High Arginine; Means in the same column with different superscript significantly differ (P<0.05).		

Table 2: Effect of oral administration of propylene glycol, L-arginine or both on mean peripheral progesterone (ng/ml) of Najdiewes.

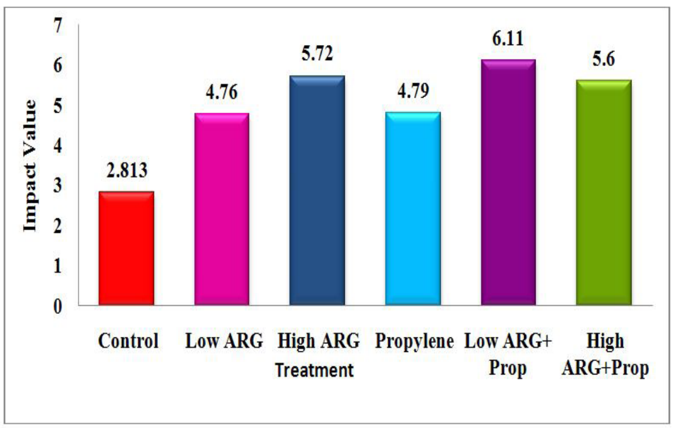

Figure 2: Effect of propylene, Arginine or both on the impact factor of lamb crop of Najdiewes.

Discussion

Najdisheep is one of the main indigenous breeds in the gulf area which mainly originated in the mid-region of Saudi Arabia. Lack of forages and feedstuffs in the desert are costly on the productive and reproductive performance of sheep herds. Moreover, such a breed is characterized by twin pregnancies and low birth weights. The main focus of the present study was to look for a feasible way to augment the pregnancy, enhance fetal growth and development and get better lambs with high survivability. Several studies have stressed on the impact of L-Arginine as a Nitrous Oxide (NO) precursor which increases the angiogenesis of placenta during pregnancy resulting in more blood flow to the fetuses [18-21]. The best value for providing pregnant Najdi ewes with L-Arginine was found to be when offered at an early rather at late stage of pregnancy which was explained from the point of enhancing the vasodilatation of the placenta to provide more nutrients for embryos at the maternal recognition of pregnancy and beyond [6].

Propylene Glycol (PG) is considered a good source of blood glucose given to treat ketosis in the early postpartum in high milk-producing cows[22,23], however none of the studies referred to its role when combined with amino acids/protein or compared to protein source on fetal growth and development during late pregnancy. A proportion of PG that is administered is absorbed from the rumen whilst the remainder is dehydrated in the rumen resulting in the production of propanal [24].In the current study, the researcher has focused on the late stage of pregnancy (i.e. third trimester) as the growth of fetus (es) requires more nutrients for the linear growth and development during this critical stage. Whether these nutrients are mainly of carbohydrate or protein origins was an open question. Drenching ewes with the recommended dose of PG (50g/hd/d) enhanced their pregnancy, resulting in not only an increase in lamb birth weight by 53% over the control but also gave better survival (83.3%) than control (75%). The mechanism of action of propylene glycol is based on lowering the Non-EsterifiesFatty Acids (NEFA) and Beta Hydroxy Butyrate Acid (BHBA) levels in blood as well as on increase of insulin and glucose levels which accelerates metabolic processes[8,14,25,26]. In the present study apparently the addition of PG to a high dose (75 mg/kg/day) of L-Arginine didn't show beneficial effects above giving the high Arginine alone (i.e. impact value of 5.6 for HAP versus5.72 for HA). Even though both surpassed the control, it has been found an equal privilege for both Low Arginine (LA) and propylene (P) on the final outcome of lamb crop (i.e. impact value 4.76 and 4.79 for LA and P, respectively compared to 2.813 for control). The best treatment (LAP) in the current study on neonatal parameters culminating in the highest impact value was the mixture of PG with the low dosage of L-Arginine (LAP). The percent of increase in lamb birth weight in LAP ewes approached 96% over the control lambs. This innovative finding open a new way for studies of using various combinations of carbohydrate sources with L-Arginine. The apparent drawback of giving High Arginine (HA) per se was the irregularity of the maternal P4 profile which disappeared in case of mixing it with propylene (HAP). The possible reason for such irregularity is because in case of HAP the total amount of L-Arginine given to an ewe during treatment was reduced to 50% of that given to HA ewes.

Apparently, administration of 75 mg L-Arginine/ head/day intervened with the corpus luteum function through the process of P4 synthesis. When L-Arginine was given at 75 and 150 mg/kg/d for 56 days at an early or late stage of gestation of ewes the P4 level either was maintained or decreased than the control values [6]. The possible reason for the increase in P4 in the present study when low but not high L-Arginine was given is that the low level of L-Arginine in the current study was half that in the previous study (37.5 mg/kg/d *vs* 75 mg/kg/d). The decrease in P4 secretion in Propylene-supplemented ewes (P) might be ascribed to that the dry matter intake (which is not determined in the current study) was reduced when PG was given to dairy cows especially during prepartum period due to its low palatability[27,28].Indicated thatnegative energy balance can reduce progesterone secretion, prolong postpartum anestrous, and interfere with cowpregnancy[29,30]. In a study by[31]for investigating the effects of short term supplementation of estrous- synchronized dairy heifers with PG (150 g per day for 13 days), they found significant increase of progesterone, insulin and small ovarian follicles. The schedule of estrous synchronization regimen in addition to the short term treatment with PG to these large body weight heifers (i.e. total dose of 1950 g PG/ heifer) might have a stimulatory impact on the corpus luteum. Apparently, in the present study the long term treatment (i.e. total of 2800 g PG/ewe) might be directed towards the metabolic requirements of the mother and fetus rather than to the ovarian function and well-established corpus luteum. In a human study investigating effects of protein supplementation during pregnancy in undernourished women,[32]reported a 31% rescue of Intrauterine Growth Restriction (IUGR). The importance of a balanced maternal diet is emphasized, especially in terms of carbohydrate quality in pregnancy and lactation, for the prevention of diet-induced adiposity and associated metabolic disruptions in the offspring [33].

In conclusion, at late pregnancy in ewes there must provide a supplement of a mixture of simple carbohydrate (i.e. propylene glycol) as glycogenic factor and a source of amino acids (i.e. L-Arginine) at a dose of 37.5 mg/head/day to facilitate the function of each other for the sake of health of mother and growing fetus (es). Although the less Number of ewes utilized in the current experiment, meanwhile a longitudinal study must be persuaded with a larger number of pregnant females to consider not only the fetal health and wellbeing but also the metabolic changes occurring during pregnancy in mother.

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