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Effect of L-Carnitine Supplementation on Oxidative Stress, Metabolic and Hormonal Profile in Pregnant Rabbit Doe

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Key words
L-carnitine, Rabbit doe, Oxidative stress, Hormonal profile, Pregnancy, Water

ABSTRACT

Several variables, such as hematology and hormone profile, influence the physiological state of pregnancy and throughout the various phases of pregnancy, the female reproductive system needs energy. Fetal health and production are negatively impacted by the body's inability to obtain enough energy at any point throughout pregnancy. Therefore this study was designed to explore the influence of dietary L-carnitine on oxidative tension, metabolic and hormonal aspects in pregnant rabbit doe. Twenty-four healthy doe of twelve months of age with a body weight of 1.5 kg were evenly divided into four groups of six does each. L-carnitine was supplied via drinking water at the rate of 0 (G-0), 100 (G-1), 150 (G-2), and 200 mg/liter (G-3) at the time of initiation of mounting throughout the experiment. The results demonstrated ($p<0.05$) reduced cholesterol, triglycerides, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) levels and higher glucose levels in the G-3 group during all trimesters (1st, 2nd and 3rd) as compared to other groups. Malondialdehyde (MDA) level decreased ($p<0.05$) in the G-3 group during 1st trimester of pregnancy, however, did not diverge among all the groups during 2nd and 3rd trimesters. Triiodothyronine (T3) and thyroxine (T4) were increased ($p<0.05$) while cortisol decreased ($p<0.05$) in the G-3 group during the 1st, 2nd, and 3rd trimester than the remaining groups. It is concluded that administration of L-carnitine in fresh water at the rate of 200mg/liter during pregnancy may reduce the serum oxidative stress and improve the biochemical profile in rabbit doe.

INTRODUCTION

Pregnancy is a physiological condition that is affected by several factors including hematology and hormonal profile (Chandra and Gibbs, 2013). The female reproduction system requires energy during different stages of pregnancy and impairment of energy supply to the body at any stage of pregnancy results in lower production and fetal health (Saikumar *et al*., 2013). Nutrition plays a vital role at the time of conception and energy supply is needed at different stages of pregnancy to fetus and dam (King, 2012). Normal pregnancy needs high metabolic demand and high requirement of tissue oxygen, depending on specific biochemical pathways for the production of cellular energy. The mitochondrial antioxidant enzymes are used as a regulator for oxidative process and they inhibit the activity of oxidant. When there is inequity among the ROS cells and antioxidants, oxidative stress is

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created (Castillo *et al*., 2003). The process of pregnancy is associated with increased metabolic demand and oxidative stress, which may affect the health of the newborn.

Carnitine is derived from the Latin word called *carnus* or fleshy tissue, isolated from meat (Rebouche, 2004). In animals, the biosynthesis of carnitine occurs mostly in vital organs such as excretory organs and hepar from its precursor called lysine and methionine and is present essentially in all biological units of the mammalian species (Golzar-Adabi *et al*., 2006). L-carnitine can get better fatty acid usage through the β-oxidation and therefore increases the availability of energy for reproduction, through the synthesis of carnitine-palmitoyl-transferees 1 and 2 (Woeltie *et al*., 1990). Addition of L-carnitine elevates plasma triiodothyronine (T3) levels (Buyse *et al*., 2001). Elgazzar *et al*. (2012) depicted that adding L-carnitine at different levels improved cholesterol profile, elevated thyroid hormone efficiency increased plasma glucose concentration, and decreased liver enzymes in New Zealand rabbits. A negative correlation has been observed between lower energy intakes of the rabbit with reproductive phenomenon in the female. The availability of energy from ingredients such as glucose, fatty acids cholesterol, triglyceride, total protein, albumin, calcium, and phosphate, has a direct impact on the reproduction of most female rabbit doe (Bioti, 2004). Hematology and serum parameters are not only important investigative tools in veterinary practices but also applied in various mammalian species including rabbits for dietary and metabolic requirements for different physiological conditions. Also, hormones are crucial in the vital body functions and development of various mammalian corpse organs (Young *et al*., 2004). Previous studies reported that there is no difference in hematological parameters during pregnancy (Brewer, 2006). Since, for differences in hematological and biochemical profile, these mammalian species are usually used as an experimental model. Therefore, this study was designed to establish the possible effect of dietary supplementation of L-carnitine in pregnant rabbits doe to reduce stress and enhance physio-chemical status during pregnancy.

MATERIALS AND METHODS

Experimental animals

The research project was executed at Rabbitry, The University of Agriculture, Peshawar. A total of 24 experimental rabbits of twelve months of age and the same body weight were used for the experiment. Rabbits were not crossed or natural services were not provided. The standard enclosure was used for their rearing at the Rabbitry. Standard protocols were adopted for feeding and watering during the rearing period. The provision of the water was twice a day. The green fodder and concentrate ration were used for feeding. Experimental rabbits were spread into four groups, having the same number of rabbits. One was the Control (G-0) group, while G-1, G-2, and G-3 groups contained L-carnitine via drinking water at the rate of 100, 150, and 200 mg/L, respectively.

Breeding

The male was provided in each group to initiate the reproduction process. Standard procedures including abdominal palpation for evaluation of positivity of gestation/ conception were used (Gill *et al*., 2004). Upon positive conception, specimens were collected during three different stages of gestation (first, second, and third trimester). Five ml blood was collected from each doe from the ear vein and centrifugation was carried out at 4000 for 20 min to split serum. Specimens were kept at -20°C for further investigation.

Biochemical analysis of blood

Standard protocols were used for the assessment of oxidative stress Okhawa *et al*. (1979). A spectrophotometry was done to assess the malondialdehyde (MDA) level in the experimental specimen. The procedure relied on the concentration of abridgment of two moles of thiobarbituric acid with one molecule of malondialdehyde consequential in the exclusion of O_2 moles of H_2O to have a TBA color. The standard procedure of Okhawa *et al*. (1979) was used and the results were interpreted using Biosystem, BTS-330, biosystem, Barcelona, Spain. The cortisol levels were determined with a marketable accessible ELISA kit (California USA). Glucose was determined in the specimen using a marketable accessible kit (Singapore Bioscience PTE Ltd). Commercially available kits (Tech Diagnostic Technologies, Spain) were used for the appraisal of cholesterol and triglycerides. aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined by using a commercially accessible kit (RANDOX B, I234, Foster City, CA, USA).

Metabolic hormones (Triiodothyronine and Thyroxine)

Triiodothyronine (T3) and thyroxine (T4) were appraised in specimens using a marketable accessible kit (Bio check, Inc. 323 Vintage Park Drive, Foster city, CA, USA). The assay was done according to the manufacturer's instructions. Briefly, 50 µl specimen and 50 µl each standard, antibody reagent in a microplate, jumble, and keep warm for one hour then add 50 µl standard, wash, and add 100 µl TMB reagent followed by incubating again for 20 min lastly add 100 µl stop solution to each well, mix and read on Eliza reader or microliter reader.

Statistical analysis

Standard methodology was utilized for the statistical evaluation of the current findings using ANOVA (Steel and Torrie, 1997). Furthermore, DMR assessments were done to establish the level of significance among different treated groups at 0.5 (Duncan, 1955).

RESULTS

Table I indicates the result of serum cholesterol in pregnant rabbits. A significantly low level of serum cholesterol was observed in the G-3 group during the 1st, 2nd, and 3rd trimester. Further, the cholesterol concentration decreased significantly in group G-2 and G-3 groups. A significantly decreased level of serum triglycerides was observed in in G-3 group during the 1st, 2nd, and 3rd trimesters. Furthermore, a significant lowering level of Triglyceride was noted in the last trimester followed by the second trimester. Serum glucose level was recorded significantly high in the G-3 group during the 1st, 2nd, and 3rd trimester. Serum AST and ALT levels were calculated significantly lower in the G-3 group in all three trimesters. During 2nd and 3rd trimesters, ALT decreased significantly in the G-2 group. Additionally, during 3rd trimester, ALT level was reduced significantly in all treated groups (G-1, 2, and 3). The results regarding the intensity of serum metabolic hormone concentration at different trimesters are shown in Table II. It was observed that serum MDA decreased ($P<0.05$) in the G-3 group in 1st trimester but during 2nd and 3rd trimester MDA concentration did not differ significantly among all the groups. Serum cortisol, T3, and T4 had a ($p<0.05$) lowered level in the G-3 group than the other groups during the 1st, 2nd, and 3rd trimester.

Table I. Level of serum metabolite in pregnant rabbit at different stages of pregnancy.

Parameters	Group	1st trimester	2nd trimester	3rd trimester
Cholesterol (mg/dL)	G-0	71.12±4.15^{a}	67.40±2.13^{a}	73.04±0.70^{a}
	G-1	66.88±2.22^{a}	59.88±2.29^{a}	55.37±1.88^{b}
	G-2	58.51±2.08^{ab}	50.91±1.00^{b}	49.39±0.86^{c}
	G-3	51.64±2.47^{b}	44.70±0.97^{b}	41.97±0.53^{d}
	P value	0.0322	0.0422	0.0165
Triglycerides (mg/dL)	G-0	134.49±2.81^{a}	131.15±5.09^{a}	128.24±3.52^{a}
	G-1	121.99±7.70^{ab}	118.75±1.39^{b}	112.37±7.30^{ab}
	G-2	115.67±5.68^{ab}	106.20±4.86^{bc}	105.89±1.25^{b}
	G-3	106.29±5.24^{b}	96.50±5.31^{c}	98.84±1.92^{b}
	P value	0.0023	0.0433	0.0221
Glucose (mg/dL)	G-0	118.47±1.87^{a}	114.35±2.20^{a}	116.97±4.04^{a}
	G-1	121.94±2.04^{b}	125.02±2.43^{b}	126.93±1.56^{b}
	G-2	142.56±2.27^{c}	146.28±1.70^{c}	148.56±2.20^{c}
	G-3	161.87±1.99^{c}	167.64±3.00^{c}	172.23±1.05^{c}
	P value	0.0021	0.0041	0.0026
AST (U/L)	G-0	29.103±1.59^{a}	30.69±2.15^{a}	31.98±0.90^{a}
	G-1	28.103±1.55^{a}	25.49±2.05^{ab}	23.82±1.72^{b}
	G-2	19.897±0.670^{b}	16.747±0.98^{bc}	14.64±0.94^{c}
	G-3	12.400±0.27^{c}	9.78±0.49^{c}	8.87±0.59^{d}
	P value	0.0243	0.0352	0.0213
ALT (U/L)	G-0	37.75±2.58^{a}	42.280±0.45^{a}	42.57±6.01^{a}
	G-1	20.02±2.30^{b}	17.25±2.22^{b}	15.21±2.83^{b}
	G-2	16.73±2.47^{ab}	12.64±0.58^{c}	9.56±1.62^{b}
	G-3	11.45±1.80^{c}	9.21±2.19^{c}	6.66±1.39^{b}
	P value	0.0142	0.0242	0.0321

Means in the column having different superscripts are significantly different at α=0.05. G-0 (Control) while G-1, G-2 and G-3 contained L-carnitine via drinking water @ 100, 150 and 200 mg/L, respectively. AST, Aspartate amino transferase; ALT, Alanine amino transferase.

Table II. Intensity of serum metabolic hormones concentration at different trimester.

Parameters	Group	1st trimester	2nd trimester	3rd trimester
MDA (nMol /mL)	G-0	6.27±7.50^{a}	6.12±3.33	6.14±8.81
	G-1	6.26±0.01^{a}	6.11±8.81	6.12±5.77
	G-2	6.25±0.01^{ab}	6.11±5.77	6.11±0.55
	G-3	6.24±6.08^{b}	6.11±8.81	6.10±5.77
	P value	0.0312	0.0814	0.0761
Cortisol (nMol /mL)	G-0	10.37±0.21^{a}	11.52±0.24^{a}	11.09±0.45^{a}
	G-1	7.55±0.28^{b}	7.74±0.34^{b}	8.74±0.14^{b}
	G-2	6.55±0.62^{b}	6.45±0.05^{c}	7.20±0.45^{c}
	G-3	5.27±0.28^{b}	5.50±0.12^{d}	4.55±0.28^{d}
	P value	0.0223	0.0341	0.0212
T3 (ng/dl)	G-0	22.42±0.00^{a}	22.06±0.00^{a}	22.06±0.20^{a}
	G-1	23.67±0.33^{b}	23.76±0.33^{b}	24.72±0.10^{a}
	G-2	25.09±0.33^{c}	25.09±0.33^{c}	24.83±0.24^{ab}
	G-3	26.42±0.00^{d}	25.82±0.00^{c}	25.95±1.26^{b}
	P value	0.0142	0.0325	0.0183
T4 (ng/dl)	G-0	33.96±0.18^{a}	34.98±0.25^{a}	36.09±0.19^{a}
	G-1	33.44±0.05^{ab}	35.99±0.24^{ab}	36.03±0.26^{b}
	G-2	34.23±0.85^{ab}	34.56±0.61^{b}	33.85±0.16^{c}
	G-3	35.05±0.06^{b}	35.35±0.25^{b}	40.02±0.19^{c}
	P value	0.0432	0.0212	0.0331

Means in the column having different superscripts are significantly different at α=0.05. MDA, Malondialdehyde; T3, Triidothyronine; T4, Thyroxine. For group description, see Table I.

DISCUSSION

Our investigation demonstrated that blood metabolites have been enhanced with the provision of L-carnitine addition during different trimesters of pregnancy in rabbits. Hence, the result obtained herein is very much fundamental to determining the dietary requirements of the experimental rabbits during different physiological stages of normal pregnancy to boost their reproductive performance to increase their productivity. The finding obtained in the current research project demonstrated the obvious reduction in serum metabolites during different stages of normal pregnancy with L-carnitine addition in comparison with the control group. Our results are in line with Elgazzar *et al*. (2012) who demonstrated that L-carnitine is associated with a discernible reduction in serum cholesterol. However, the functional mechanism through which this reduction occurred is not yet clearly known. However, this reduction in serum cholesterol might be due to the higher oozing out of steroids from bile or might be a translation of cholesterol into bile acids. Likewise, the reduced serum triglyceride was noted during the current investigation. The reduction in serum triglyceride might be ascribed to higher utilization of protein with lipid and its enzymes and lipid oxidative physiology. Dietary L-carnitine has been related to oxidation (Tanaka *et al*., 2004). This might be the possible reason for the reduction of triglyceride during these physiological stages of normal pregnancy. During the current study, it was observed that L-carnitine substantially increased in T3 and T4 concentration in treated groups in contrast with the control group. The data generated here about T3 and T4 are in agreement with research conducted on chicken and rabbit previously (Buyse *et al*., 2001; Elgazzar *et al*., 2012) who used chicken and rabbit as an experimental model demonstrated that L-carnitine addition significantly increased the thyroid hormones concentration in chickens and rabbits respectively. The exact mechanism through which L-carnitine increases thyroid hormone concentration is not known, however, Benvenga *et al*. (2004) suggested that L-carnitine causes the accumulation of thyroid hormones in peripheral blood by inhibiting their entry into the cell.

During the current research trial, serum glucose concentration was considerably high when judged against a control group. The possible factor for the higher glucose concentration during the current trial with the addition of L-carnitine might be associated with an increase in the T3 and T3 concentration during these physiological stages of normal pregnancy. These have been concurrent with earlier research elucidations. Greenwood *et al*. (2001) and Elgazzar *et al*. (2012) demonstrated the substantial boosting up in the plasma glucose level with animals whose feed was supplemented with L. carnitine. For that reason, the high level of glucose that was observed in these reports including our investigation might be associated with the oxidation of augmented fatty acid followed by the lowering of gluconeogenic precursors (Elgazzar *et al*., 2012). Conversely, L-carnitine reduced glucose concentration in chicken plasma in the study of Buyse *et al*. (2001) probably due to different animal species, doses of L-carnitine, and different experimental protocols. The L-carnitine addition radically reduced cortisol concentration in the present study. Martin *et al*. (2003) suggested that this hormone is associated with the stimulation of many biological processes and functional mechanisms including the synthesis of glucose etc. Besides these stimulatory functions, it has the facilitation capacity of the activation of glycogen phosphorylase, which is necessary for epinephrine to affect glycogenolysis.

The serum MDA value significantly decreased during the first trimester in does fed L-carnitine at the rate of 200 mg/L of water. A gestation is a functional event and condition wherein female subjects are exposed to oxidation or related tension owing to disparity involving many aspects of oxidation and anti-oxidation mechanisms (Saikumar *et al*., 2013). Adiga and Adiga (2009) suggested that increased nutrient essentialities as well as other related factors are fundamental during the events of gestation for organ oxygenation as well as organogenesis. This event of gestation is associated with ROS production due to the provision of essential metabolic aspects. Saikumar *et al*. (2013) suggested a possible factor for the reduction of MDA. It has been suggested that amplified ATP production in the feto-maternal interface at the placental structure and high demand for oxygenation for growing tissue during the gestation period might be the potential factors for the reduction in MDA. Serum AST and ALT levels have been found substantially lowered in our current investigation using L-carnitine. Our results regarding AST and ALT are similar to the study carried out by Sanjay and Singh (2010) on rats. Furthermore, the results obtained in the current research project are in line with the report of Ahmed *et al*. (2010) and established a marked reduction in these two metabolic indicators during experimental work on L-carnitine. Though the functionality of these two indicators is not yet very clear, however lowering of these values might be indicative of some damaging effect on the liver and other related organs. Hence it seems reasonable that L-carnitine might have a potential ameliorative role in hepar physiology.

CONCLUSION

L-carnitine supplementation in rabbits reduced

oxidative stress, cholesterol profile, and liver function enzyme concentration while increasing glucose concentration and improving metabolic hormonal profile during pregnancy at the rate of 200 mg/L. L-carnitine is recommended to be tested on the body weight of neonates in other domestic animals during pregnancy.

DECLARATIONS

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IRB approval

The study was approved by the Board of Studies (BOS) meeting of the College of Veterinary Science, The University of Agriculture Peshawar (No.1304/A.H, dated,04/05/ 2023).

Ethical statement

This study was approved by the Animal Welfare and Care Committee of the Faculty of Animal Husbandry and Veterinary Sciences, The University of Agriculture, Peshawar, Pakistan, and all the measures and tools were considered to minimize the pain and discomfort of a rabbit during the conduction of this experiment.

Statement of conflict of interest

The authors have declared no conflict of interest.

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