

THE RELATION BETWEEN OXIDATIVE STATUS, MILK QUALITY AND CONCEPTION RATE IN DAIRY GOATS SUPPLEMENTED WITH VITAMIN C

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ABSTRACT

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Pregnant Agamy does were used in this experiment to evaluate the effects of Vitamin C (Vit C) supplementation as antioxidant agent to improve the performance of dairy goats during the evidence of oxidative stress (OS) in peri-parturient period. The does under experiment (n = 16) were assigned randomly into control and supplemented groups (n = 8 / group). The control group fed control diet without supplement and the supplemented group fed control diet plus Vit C 75mg/kg of body weight. Dietary supplementation began approximately one month before the expected kidding date till two months post-kidding. Compared to the control does, it was noticed that Vit C supplementation significantly increased (P<0.05) the conception rate (62.5 % vs. 25 %), the weaning weight (WW) (7.21 vs. 5.00 kg) as well as the average daily gain rate (DGR) (53.94 vs. 34.85 g/d) in the kids of the Vit C supplemented group. Vit C supplementation significantly (P<0.05) increased serum level of T₃ from day 45 to day 60 and of T₄ from day 30 to day 60 post-kidding. Meanwhile, serum cortisol level was significantly decreased (P<0.05) from day 30 to day 60 post-kidding in the Vit C supplemented group. Also, there was a significant increase (P<0.05) in serum total antioxidant capacity (TAC) from day 15 post-kidding and glutathione peroxidase (GPX) from day 30 post-kidding till the end of the experiment in the Vit C supplemented group as compared with the control one. Meanwhile, there was a significantly decrease (P<0.05) in serum Malondialdehyde (MDA) level from day 45 post-kidding and nitric oxide (NO) level from day 15 post-kidding till the end of the experiment. Concerning the effect of Vit C supplementation on milk quality, it was found that Vit C supplementation significantly (P<0.05) improved most of milk components especially fat, lactose, milk urea level (MUL), total solids (TS) and solid not fat (SNF); meanwhile there was a non-significant increase in protein % all over the experiment period. Moreover, both somatic cell counts (SCC) and the infection rates represented by the total bacterial count (TBC) were significantly (P<0.05) decreased in the Vit C supplemented group when compared with the control one. It was concluded that Vit C supplementation in dairy does around the peri-parturient period can improve the unfavorable effects of OS; this reflected on their milk quality and post-kidding conception rate and enhancement of growth rate as well as weaning weight of their kids.

Key words: *Oxidative status, Milk quality, Conception rate, Dairy goats, Vitamin C.*

INTRODUCTION

Pregnancy and lactation are physiological stages considered to induce metabolic stress (Drackley, 1999). During this period, dairy animal

experienced moderate OS, which plays a key role in several pathological conditions connected with animal production, reproduction and welfare (Lykkesfeldt and Svendsen, 2007 and Celi *et al.*, 2008). In late pregnancy and early lactation negative

energy balance may be the reason for development of OS (Roche *et al.*, 2000); as heavy milk yielders have higher metabolic rate to derive energy involving oxidation reactions to meet the demand of production, these processes result in the formation of free radicals. To neutralize these free radicals, the body employs the system of endogenous antioxidants; when the capacity of antioxidant defense is exceeded by the production of reactive oxygen substances (ROS), resulted in the development of OS. Increased OS in peripartum period of dairy cows and goats play an important role in development of metabolic diseases like ketosis, post-parturient haemoglobinuria and post-parturient paresis (Celi *et al.*, 2010). A number of recent studies have reported variable levels of OS during the peripartum period in dairy goats (Celi *et al.*, 2008 and 2010). It seems that ROS and antioxidants may be involved in some relevant physiological functions such as milk yield (Gabai *et al.*, 2004).

Free radicals are natural final products of the intensive metabolism in cells in high-yielding dairy cows. When the disturbing of homeostasis occurs, the oxidative processes lead to OS which causes mastitis that can cause the reduction of milk yield and unfavorable changes in the milk composition. Also, OS is associated with retaining placenta after calving and disrupting the activity of the corpus luteum (CL) that affects the reproductive functions. The active immune response to inflammation leads to an increase in the secretion of other molecules having an adverse effect on embryo survival (Strzałkowska *et al.*, 2009a and Józwick *et al.*, 2012c). Lipid peroxidation is one of the important consequences of OS; it can be used for the evaluation of OS severity (Kumaraguruparan *et al.*, 2002 and Halliwell and Whiteman, 2004). MDA is a degradation product of lipid peroxidation and is a marker of OS (Castillo *et al.*, 2005). During inflammatory diseases, high levels of NO react with superoxide anions leading to formation of peroxynitrite radical causing lipid peroxidation which may inhibit the activity of some antioxidant molecules as GPX leading to OS (Atroschi *et al.*, 1996 and Weiss *et al.*, 2004). Therefore it might be beneficial to supplement dairy animal with antioxidants (Pedernera *et al.*, 2009). Antioxidants are the substances that can protect cells from damage caused by ROS (Gutteridge, 1995). Ascorbic acid (Vit C) is one of the strong reductant and radical scavengers (Rice, 2000); it performs antioxidant function either by quenching various free radical species directly or by reducing membrane bound oxidized vitamin E at the membrane surface.

However, OS continues to be a problem in transition dairy animal. Innovative approaches are needed to enhance the antioxidant defense mechanisms of dairy animal during times of increased metabolic demands.

On the reverse of other ruminants that are grown in our country, goats were placed on the last interests within the livestock, although it has the ability to provide high quality milk that used for production of certain types of highly expensive dairy products. Further, there are many experiments conducted to study the effect of ascorbic acid (Vit C) as an antioxidant at various stress conditions in different species. But the reports were meager on the effect of ascorbic acid on metabolic stress and OS parameters of goats during peripartum period. Hence, this study was undertaken to assess the effect of ascorbic acid supplementation on dairy goats during advanced pregnancy and early lactation period by analyzing of some blood biochemical markers, quality characteristics of milk (physical, chemical and bacteriological indicators) and some reproductive performance parameters.

MATERIALS and METHOD

Experimental Design:

16 pregnant Agamy does were used in this experiment, in the 4th month of pregnancy of 2-3 years of age. The does were apparently healthy by physical examinations. These animals were randomly divided into two groups of 8 animals each. All animals were housed in an open sheds, under conditions of natural day light and temperature. Does in the group 1 (control group), were fed a standard total mixed ration according to the management of the farm, feed ingredients were; (ground yellow corn 38%, soybean meal 28.35%, wheat bran 30.2 %, lime stone 0.75%, sodium bicarbonate 1.5 %, zinc methionine 0.1%, monophosphate 0.3%, antitoxin 0.1%, yeast culture (*Saccharomyces Cerevisiae*) 0.2% and mineral mixture 0.6%) in addition to barseem (alfa alfa). Does in the second group were fed ration plus 75 mg Vit C /kg of body weight /day (Vit C supplemented group) (Vit C; powder supplied by the Egyptian Arabian Co. of feed additives and vitamins), this supplementation started from the last month of pregnancy up to 60 days post-kidding, Water was offered ad-libitum.

Blood sampling and measurements:

Blood samples were taken in heparinized tubes for plasma separation and in plain tubes for serum separation from all does through jugular vein in the morning (before watering and feeding). Blood samples were taken every two weeks, 30 days before kidding, then every two weeks till 60 days post-kidding and after stopping of Vit C supplementation. Serum and plasma were separated by centrifugation at 3000 rpm for 15 min, and stored at -20^oC for further analyses.

All hormonal analyses were performed by using ELISA 96 micro-wells kits (Mono bind Inc. Lake forest, CA92630.USA); Total tri-iodothyronine (T_3): Braverman (1996), total thyroxin (T_4): Muzzaffari and Gharib (1998) and cortisol: Buritis and Ashweed (1994).

Plasma was analyzed colorimetrically by commercial kits for ascorbic acid (Vit C) (Harris and Rays, 1935) and serum was analyzed for, free radicals; lipid peroxidase (MDA) (Ohkawa *et al.*, 1975) and NO (Rajaraman *et al.*, 1998) and for antioxidants: TAC (koracevic, 2001) and GPX (Pagila and Valentine, 1967).

Kids Performance:

All does of the both groups were labored normally. Newly born kids were numbered, sexed, weighed on day of birth to determine birth weight, (BW), and then on day 90 of age to determine the weaning weight, (WW) also daily gain rate (DGR) were calculated.

Reproductive performance:

Three fertile bucks were introduced for 30 minute/day with all does of the two groups for natural mating beginning from day 30 till day 60 post-kidding. Pregnancy was diagnosed at day 60 post-kidding by ultrasound scanner (200 pies Medica co. - Netherlands, Holland). The number of conceived does was recorded in each group for calculation of conception rate.

Milk sampling and analyses:

Composite milk sample was collected (after sterilization of the udder halves with alcohol 70%) from each dairy doe in the two groups every two weeks beginning from day15 till day 60 post-kidding. Samples were immediately transported into the laboratory on ice and then were analyzed for bacterial counting, isolation and identification of the major pathogen germs. Also, the samples were analyzed using Lactoscan SLP (Bulgaria) to estimate fat, protein, lactose, TS and SNF and SCC was determined using the Nucleocounter SCC-100 (Chemometric Nucleocounter Family, Denmark). MUL was estimated using colorimetric kits from Spectrum Diagnostic Company, Egypt, according to Batton and Crouch (1979).

Bacteriological analyses:

Milk samples were processed under complete aseptic conditions with the aim to isolate most common bacteria and to determine the total bacterial count; TBC (colony forming unit; CFU/ml) by using plate count method (PC) according to International Dairy federation (IDF, 1991). To isolate bacteria from milk sample, 0.01 ml was sowed on blood agar, mannitol

salt agar, mac Conkey agar and Edward's media and incubated at 37°C for 24-48 h. Identification of isolates was made after examination of cultural features, morphological features using Gram stain and biochemical identification according to Quinn *et al.* (2011).

Statistical analysis:

All data were subjected to statistical analysis according to Sendecor and Cochran (1989) and analyzed by one way ANOVA implying a completely randomized design using Costat version 3.03. Conception rate was calculated using Hypothesis test Proportions Microsoft copyright (C) 1984 by Eco soft, Inc. [data program].

RESULTS

Results depicted in Fig. (1) indicated that there was a highly significant ($P<0.05$) increase in conception rate (62.5%) in the Vit C supplemented group as compared with the control one (25%).

Table (1) showed that, kids' average BW, WW and DGR were affected by Vit C supplementation to their dams during late pregnancy and early lactation. Kids of Vit C supplemented group had BW similar to that of the non-supplemented control one, meanwhile these kids were significantly heavier ($P<0.05$) at weaning and showed a significantly higher ($P<0.05$) DGR than that of the control dams.

Data analysis showed that significantly ($P<0.05$) increased in the plasma Vit C levels of the supplemented group at one and two month post-kidding when compared with that of the non-supplemented control one Table (2).

Data analysis Table (3) showed that serum T_3 levels were increased significantly ($P<0.05$) at days 45 and 60 in the supplemented group and non-significantly at days 15 and 30 post kidding, meanwhile serum T_4 was significantly ($P<0.05$) increased from days 30 post kidding till the end of the experiment. On the other hand, serum cortisol levels was significantly ($P<0.05$) decreased from days 30 post kidding till the end of the experiment and non-significant on day 15 post-kidding in Vit C supplemented group as compared with the control one.

Tables (4&5) showed that, the TAC was significantly higher ($P<0.05$) in Vit C supplemented group as compared to the control one from day 15 post kidding till the end of the trial and showed that Vit C supplementation significantly ($P<0.05$) decreased the serum NO levels from day 15 and MDA levels from day 45 and significantly increased GPX levels

($P < 0.05$) from day 30 post-kidding till the end of the trial as compared with that of the non-supplemented control one.

All the previous blood parameters were non-significantly differed at 2 weeks post- stopping the Vit C supplementation in Vit C supplemented group when compared with the control one except GPX levels which still significantly ($P < 0.05$) increased in Vit C supplemented group.

Concerning milk composition Table (6) cleared that Vit C supplementation significantly ($P < 0.05$) increased fat % on days 30, 45 and 60 post-kidding, meanwhile the increment was non-significant on day 15 post-kidding. Also, there was a non-significant increase in the protein % due to Vit C supplementation all over the experiment time. Moreover, lactose %, TS% and SNF% were significantly increased ($P < 0.05$) in milk of Vit C

supplemented group as compared with that of the control one all over the experiment. On studying the effect of Vit C supplementation on MUL, the result showed that there was a non-significant decrease in its level on day 15, while the decrease was significant ($P < 0.05$) on days 30, 45 and 60 post- kidding. Moreover, both SCC and TBC were highly significantly ($P < 0.05$) decreased in Vit C supplemented group as compared with that of control one.

The most frequently isolated bacteria in both control and Vit C supplemented groups were CNS and environmental streptococci and the lowest ones were *E.coli* and *S.aureus*, Table (7). These results declared that Vit C supplementation did not affect the type of bacteria but significantly affected on their infection rates and bacterial counts in the milk samples of the examined does along the experimental period.

Fig. 1: Conception rate as affected by dietary supplementation of does with Vit C during late pregnancy and early lactation period.

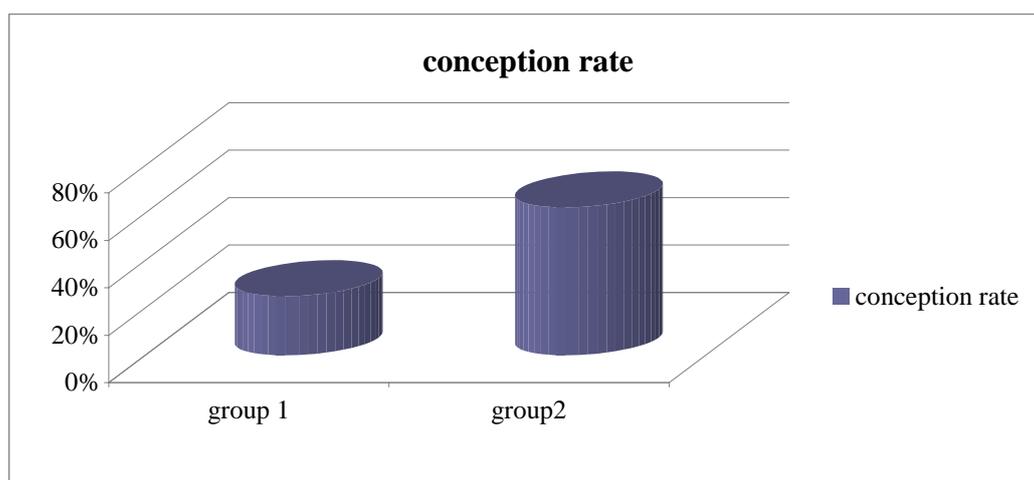


Table 1: Birth weight (BW), Weaning weight (WW) and Daily gain rate (DGR) of kids from does fed on diets with or without Vit C.

Main Effect	Weight		DGR (g/d)
	BW (kg)	WW (kg)	
Control	2.07 ± 0.16	5.00 ± 0.42 ^b	34.85 ± 3.36 ^b
Vit C	2.36 ± 0.18	7.21 ± 0.32 ^a	53.94 ± 2.89 ^a

Weaning weight adjusted at 90 days after birth.

Means with different superscripts letters in the same column are significantly different at $P < 0.05$.

Table 2: Serum Vit C concentrations ($\mu\text{g/L}$) of control and Vit C supplemented does.

Main Effect	1 Month post-kidding	2 Months post-kidding
Control	0.69 ± 0.007 ^b	0.70 ± 0.007 ^b
Vit C	0.82 ± 0.014 ^a	0.84 ± 0.068 ^a

Means with different superscripts letters in the same column are significantly different at $P < 0.05$.

Table 3: Serum T₃, T₄ and Cortisol concentrations of control and Vit C supplemented does.

	+ 15 day	+ 30 day	+ 45 day	+ 60 day	+ 75 day
T3 (ng/ml)					
Control	5.22± 0.16 ^a	4.75 ± 0.53 ^a	4.75 ± 0.15 ^b	4.45 ± 0.25 ^b	3.62 ± 0.18 ^a
Vit C	5.50 ± 0.19 ^a	5.55 ± 0.17 ^a	5.66 ± 0.22 ^a	5.52 ± 0.16 ^a	4.01± 0.17 ^a
T4 (µg/dl)					
Control	7.73 ± 0.33 ^a	7.03 ± 0.24 ^b	9.50 ± 0.33 ^b	9.75 ± 0.18 ^b	10.66 ± 0.27 ^a
Vit C	8.35 ± 0.39 ^a	8.30 ± 0.22 ^a	11.49 ± 0.41 ^a	13.05 ± 0.64 ^a	10.69 ± 0.40 ^a
Cortisol (µg/dl)					
Control	66.63 ± 1.95 ^a	67.63 ± 1.77 ^b	63.88 ± 2.81 ^b	64.88 ± 2.32 ^b	63.13 ± 2.52 ^a
Vit C	62.5 ± 1.79 ^a	52.75 ± 2.59 ^a	47.00 ± 3.06 ^a	47.63± 2.52 ^a	61.00 ± 1.53 ^a

Means with different superscripts letters in the same column are significantly different at P<0.05.
+After kidding

Table 4: Serum NO and MDA concentrations of control and Vit C supplemented does.

	-30 day	+15 day	+30 day	+45 day	+60 day	+75 day
NO (µg/ml)						
Control	0.20 ± 0.014 ^a	0.28 ± 0.031 ^b	0.18 ± 0.015 ^b	0.17 ± 0.013 ^b	0.16 ± 0.005 ^b	0.17± 0.011 ^a
Vit C	0.19 ± 0.011 ^a	0.20 ± 0.008 ^a	0.14 ± 0.004 ^a	0.13 ± 0.004 ^a	0.14± 0.008 ^a	0.18± 0.055 ^a
MDA(nmol/ml)						
Control	1.63± 0.07 ^a	1.67± 0.08 ^a	1.60± 0.08 ^a	1.57± 0.05 ^b	1.53± 0.04 ^b	1.38± 0.06 ^a
Vit C	1.62± 0.06 ^a	1.51± 0.08 ^a	1.44± 0.04 ^a	1.27 ± 0.17 ^a	1.15± 0.09 ^a	1.38± 0.18 ^a

Means with different superscripts letters in the same column are significantly different at P<0.05.
+After kidding - Before Kidding

Table 5: Serum TAC and GPX concentrations of control and Vit C supplemented does.

	-30 day	+15 day	+30 day	+ 45 day	+ 60 day	+75 day
TAC(mM/l)						
Control	0.19 ± 0.013 ^a	0.29 ± 0.019 ^b	0.23 ± 0.007 ^b	0.23 ± 0.010 ^b	0.29 ± 0.028 ^b	0.26 ± 0.012 ^a
Vit C	0.21 ± 0.015 ^a	0.34 ± 0.010 ^a	0.35 ± 0.005 ^a	0.37 ± 0.009 ^a	0.36 ± 0.010 ^a	0.26 ± 0.017 ^a
GPX(U/dl)						
Control	54.68 ± 1.62 ^a	59.08 ± 0.52 ^a	62.46 ± 1.20 ^b	60.50 ± 1.95 ^b	92.26 ± 2.14 ^b	74.58 ± 3.07 ^b
Vit C	54.67± 2.96 ^a	60.47 ± 1.24 ^a	69.35 ± 1.37 ^a	100.12 ± 3.43 ^a	102.50 ± 2.14 ^a	91.3 ± 2.71 ^a

Means with different superscripts letters in the same column are significantly different at P<0.05.
+ After kidding
- Before Kidding

Table 6: Milk components, SCC and TBC of control and Vit C supplemented does.

	+ 15 day	+ 30 day	+ 45 day	+ 60 day
Fat (%)				
Control	2.89 ± 0.11 ^a	2.36 ± 0.11 ^b	2.72 ± 0.55 ^b	2.76 ± 0.43 ^b
Vit C	3.06 ± 0.17 ^a	3.39 ± 0.13 ^a	3.33 ± 0.13 ^a	3.42 ± 0.11 ^a
Protein (%)				
Control	2.58 ± 0.25 ^a	2.72 ± 0.31 ^a	2.54 ± 0.23 ^a	2.55 ± 0.14 ^a
Vit C	2.80 ± 0.13 ^a	2.80 ± 0.08 ^a	2.75 ± 0.42 ^a	2.68 ± 0.08 ^a
Lactose (%)				
Control	3.55 ± 0.08 ^b	3.26 ± 0.05 ^b	3.03 ± 0.08 ^b	2.77 ± 0.11 ^b
Vit C	4.04 ± 0.09 ^a	3.43 ± 0.04 ^a	3.35 ± 0.07 ^a	3.33 ± 0.10 ^a
MUL (mg/dl)				
Control	21.05 ± 0.71 ^a	27.48 ± 1.51 ^b	25.62 ± 1.72 ^b	30.51 ± 0.88 ^b
Vit C	20.60 ± 0.49 ^a	17.25 ± 1.44 ^a	18.88 ± 0.43 ^a	25.44 ± 1.27 ^a
TS (%)				
Control	9.60 ± 0.34 ^b	9.20 ± 0.18 ^b	9.30 ± 0.23 ^b	9.07 ± 0.29 ^b
Vit C	10.75 ± 0.17 ^a	9.85 ± 0.46 ^a	10.35 ± 0.21 ^a	10.25 ± 0.90 ^a
SNF (%)				
Control	7.28 ± 0.16 ^b	6.47 ± 0.15 ^b	6.60 ± 0.10 ^b	6.10 ± 0.09 ^b
Vit C	7.77 ± 0.15 ^a	7.05 ± 0.16 ^a	7.06 ± 0.14 ^a	6.60 ± 0.13 ^a
SCC (x10³ cells/ml)				
Control	474.63 ± 21.44 ^b	436.13 ± 40.18 ^b	487.63 ± 30.63 ^b	343.5 ± 14.31 ^b
Vit C	242 ± 8.34 ^a	158.13 ± 6.98 ^a	155.5 ± 7.24 ^a	166.5 ± 8.63 ^a
TBC(CFU/ml)				
Control	39500 ± 1238.55 ^b	36500 ± 1490.21 ^b	33875 ± 1796.19 ^b	38750 ± 2073.35 ^b
Vit C	1375 ± 70.04 ^a	4575 ± 211.80 ^a	4600 ± 210.32 ^a	4325 ± 299.58 ^a

Means with different superscripts letters in the same column are significantly different at P<0.05.

+ After kidding

Table 7: The most common bacterial isolation in the control and Vit C supplemented does.

	+ 15 day	+ 30 day	+ 45 day	+ 60 day
Bacterial isolation				
Control	CNS (37.5%)	CNS (87.5%), <i>S.aureus</i> (12.5%), Environmental streptococci (75%), <i>E.coli</i> (37.5%)	CNS (87.5%), <i>S.aureus</i> (12.5%), environmental streptococci (12.5%), <i>E.coli</i> (12.5%)	CNS (50%), <i>S.aureus</i> (37.5%), environmental streptococci (37.5%), <i>E.coli</i> (25%)
Vit C	CNS (12.5%)	CNS (50%), environmental streptococci (50%)	CNS (62.5%), environmental streptococci (25%)	CNS (30%), <i>S.aureus</i> (12.5%), environmental streptococci (12.5%), <i>E.coli</i> (12.5%)

+ After kidding

DISCUSSION

Oxidative stress is an active field of research in veterinary medicine and has been implicated in numerous diseases process including sepsis, mastitis, enteritis, pneumonia, respiratory and joint diseases (Lykkesfeldt and Svendsen, 2007). So our work dealt with Vit C supplementation to study its physiochemical and bacteriological effects as antioxidant agent on does laid under OS in late pregnancy and early lactation (periparturient period).

Our results clearly indicated that Vit C supplementation to periparturient does was significantly increased the post-kidding conception rate as compared with the control group. This result agreed with those obtained by Howida and Maha (2013) who stated that a pre-mating Vit C supplementation to ewes during summer improved their conception rate and Haliloglu and Serpek (2000) who reported that Vit C supplementation to ewes during the breeding season improved their fertility. The increased conception rate in the Vit C supplemented group may be attributed to Vit C content in the CL which may be improved due to Vit C supplementation. Petroff *et al.*, 1997 reported that Vit C content in the CL was at its maximum level in fully mature CL remained high during pregnancy and decreased as the CL regressed. Moreover, it was found that Vit C supplementation to embryo culture medium (IVF) followed by incubation at 20% oxygen level resulted in significantly higher rates of cleavage, morula, blastocyst formation and blastocyst total cell count in sheep, which reflected the protective effects of Vit C against the oxidative damage to DNA (Natarajan *et al.*, 2010).

Many reports discussed the role of both OS and antioxidants in peripartum period. Celi *et al.* (2010b) assumed the high rate of pregnancy failure to be a consequence of insufficient communication between the conceptus and the maternal environment, as biomarkers of OS seem to be involved in this network. Concentrations of ROS may also play a major role in both fertilization and implantation of oocytes (Sharma and Agarwal, 2004). Similarly, in case of a redox balance disorder, the appearing of metabolic stress can disrupt the activity of CL and suppress the progesterone (P₄) synthesis and could lead to a premature luteolysis (Sugino, 2006 and Rizzo *et al.*, 2007); these opinions may explain the lower conception rate in the non-supplemented group in our study. One of the proposed mechanisms to explain the luteolysis effect is the generation of the ROS which cause cytotoxic effect and a consequent inhibition of P₄ synthesis (Niswender *et al.*, 2000 and Pepperell *et al.*, 2003). Miller *et al.* (1993) showed that deficiency of antioxidants resulted in a lower

muscle tone and poor uterine contraction that inhibited the transport of semen into the oviduct. Moreover, in dairy cows OS was associated with retained placenta, edema of the udder and mastitis that may indirectly affect the reproductive functions (Jóźwik *et al.*, 2012c).

Administration of antioxidants could affect fertility in two ways: Firstly, a reduction in incidence of retained placenta or uterine infections caused by peripartum administration of antioxidants could increase fertility, because pregnancy success after insemination which was reduced in cows with these disorders (López - Gatiús *et al.*, 2006 and McDougall *et al.*, 2007). Secondly, the oocyte and pre-implantation embryo were susceptible to be damaged by ROS (Favetta *et al.*, 2007; Schwarz *et al.*, 2008 and Moss *et al.*, 2009) and increasing of the antioxidant status of the reproductive tract in the post-partum period might improve the competence of the oocyte or the embryo for development.

Concerning kid performance in the second group, the increment in average DGR as well as WW of the kids may be due to the increased milk fat % recorded in their dams than that of the control group. This finding has previously been reported by Sampelayo *et al.* (2006) and Bauattour *et al.* (2008) in goats. As well as greater milk quality and quantity implied greater nutrient and energy availability for kids, which were important in the average DGR and/or number of weaned animals, mainly when multiple births were presented in a non-dairy breeds of sheep (Gavojdian *et al.*, 2013).

On the basis of the pervious experiments carried out with pre-ruminant kids, it well known that during growth, an increase in quantity of dietary fat produced a more effective utilization and higher retention of dietary protein, this phenomenon is known as the sparing effect of the fat, as the fat in the milk contributes to the energy requirements associated with protein synthesis (Sampelayo *et al.*, 2006). The obtained results for DGR were in agreement with the above comments. Furthermore, when milk is the sole source of food to the pre-weaned kids, the general condition and the average daily gain of kids are a direct reflection of the amount and the quality of milk produced by the dam (Cimen and Karaalp, 2009 and Martínez-González *et al.*, 2015). The amount of milk produced has a strong influence on kid growth, as 20–80% of the variation in WW was due to the volume of milk produced or suckled (Sibanda, 1992 and Adogla-Bessa and Aganga, 2000). Due to the fact that, a close correlation was existed between the OS and mastitis in dairy animal a superiority of oxidizing processes can indicate a subclinical inflammation of the mammary gland. The inflammation of udder during

the first three weeks after parturition, can lead not only to a reduction of milk yield and unfavorable changes in the milk composition but also, to adverse effect on the health of the entire organism (Strzałkowska *et al.*, 2009a and Jóźwik, 2010c). As Vit C affected both frequency of mastitis and severity of its symptoms and there was a significant correlation between Vit C concentrations in milk and plasma and clinical signs of mastitis (Weiss *et al.*, 2004; Weiss and Hogan, 2007) following an intramammary challenge with *E. coli*. So that Vit C supplementation during periparturient period improved the milk quantity and quality. Therefore, the linear responses on average DGR and WW observed here were in agreement with higher milk fat yielded within Vit C supplemented dams.

Hence our results showed positive effects of Vit C on thyroid hormones levels, which could be due to its direct involvement on thyroid gland or on deiodinase enzyme activity. Vit C can promote thyroid health by reducing the OS that produced either by foreign toxins or harmful free radicals during the syntheses of thyroid hormones (Ayo *et al.*, 2006). The present results were supported by many recent studies; Sivakumar *et al.* (2010), and Peepere *et al.* (2014) who noticed that Vit C supplementation significantly increased serum T₃ and T₄ levels in heat stressed goat and during certain diseases that caused excessive thyroid gland oxidations.

The higher serum cortisol level in the control does in the current study reflect the effect of high metabolic demand due to higher milk production. Lefcourt *et al.* (1993) reported that the glucocorticoids constituted an important component of many physiological functions, which included stress and metabolism. The present results agreed with Alameen *et al.* (2014) who found an increase in serum cortisol level in high producing dairy cows as compared with low producing dairy cows.

Due to the difficulty of measuring each antioxidant component separately and their interaction in the serum, several methods have been developed to assess total antioxidant capacity (TAC). The measure of antioxidant capacity was considered the cumulative action of all antioxidants present in the serum and body fluids, thus providing an integrated parameter rather than the simple sum of measurable antioxidants (Ghiselli *et al.*, 2000).

In the present findings, blood GPX and TAC were significantly higher in Vit C supplemented group than the control one. In contrast, MDA and NO were significantly lower in the same group during the transition period. However, Aitken *et al.* (2009) had reported that activities of GPX were increased during

early lactation. GPX proteins catalyze the reduction of organic hydroperoxides, lipid peroxides, and hydrogen peroxide, using glutathione as reducing agent to protect the cells from the oxidative damage resulting from normal oxidative metabolism. Furthermore, a significant correlation was recorded between antioxidant supplementation and decreased incidence of mastitis which supports our results and presumptions. Therefore, an imbalance between the increased production of ROS and the reduced availability of antioxidant defense near the time of parturition might increase OS and may contribute to periparturient disorders in dairy cows (Sharma *et al.*, 2011).

Concerning the milk composition of dairy goats in this study, it was important to measure the goat's milk component. As it was suggested that the etiology of mastitis is associated with the OS, this may be expressed by milk production and its content of lactose. Also, a positive relation between the milk lactose content and the health of the mammary gland was demonstrated (Bansal *et al.*, 2005), that approved our results. Furthermore, lactose and protein concentrations were significantly affected by the bacteriological status of the animal (Leitner *et al.*, 2007). Likewise, fat % of milk was significantly increased in the does supplemented with Vit C, this not only improved the milk quality but also increased ADG as well as WW of the kids. Further, MUL was significantly influenced by nutritional and non-nutritional factors (milk crude protein and milk yield) and reached to its peak in the mid lactation period which agreed with our results (Rapetti *et al.*, 2013). However goats produced milk of the highest quality with good TS and SNF when given the right care including good clean home, good quality feed and proper milking (Lunguru, 2008) that achieved by our results in the Vit C supplemented group.

SCC is not only considered an indicator of intramammary infection (IMI), but also a sensitive tool for analyzing its effects on milk yield, milk composition and efficiency of curd and cheese production and other factors negatively influenced by IMI; SCC of milk is considered a sensitive marker of udder health condition in goats (Raynal-Ljutovac *et al.*, 2007). As well, SCC of milk is an indicator of the OS (Suriyasathaporn *et al.*, 2006). Under the legislation of some countries, SCC must be $\leq 400,000$ cell/ml for raw milk (Voşgan, 2013), although a threshold of 10^6 cells/ml showed the best indication for IMI, high SCC does not always reflect mastitis probably due to the apocrine secretion in goats (Teleb *et al.*, 2014).

Consequently, the high SCC negatively affected the milk components including fat, lactose, MUL, TS and SNF in cow's milk and high SCC scores altered milk

composition in goats (El-wakeel *et al.*, 2010 and Teleb *et al.*, 2014) that declared in our results as by decrease SCC in Vit C supplemented dams, most of the milk components improved than that of the control one. In another investigation, a positive correlation between SCC and protein content and a negative correlation between SCC and lactose were showed (Pajor *et al.*, 2013), furthermore, SCC correlated to TBC and their results indicated that higher SCC in raw goat's milk was associated with disadvantageous milk properties, which may lead to reduced milk quality that confirmed our results.

In Europe and USA, the main regulatory mean to prevent the occurrence of zoonosis and other pathogenic bacteria and their toxins in marketed goat's milk is through bacterial count of the raw milk (Pirisi *et al.*, 2007).

The oxidative processes occurred in milk, besides lowered its nutritive value, had a negative effect on organoleptic parameters, principally taste and caused the inactivation of many biologically active ingredients contained in it. As a rule, the increase in SCC and bacteria in milk played a key role in the assessment of milk quality (Sawa *et al.*, 2008 and Bagnicka *et al.*, 2011). Therefore, the present study recommends for the periodical examination of dairy goat's milk for TBC as in bovine milk in Egypt before being processed as dairy products.

Dietary supplementation with ascorbyl-2-polyphosphate decreased milk SCC in dairy cows with mastitis induced by the endotoxin challenge (Weiss and Hogan, 2007) that greatly accepted with our results as SCC decreased in the Vit C supplemented does as compared with the control one. Moreover, antioxidants were reported to enhance the neutrophil-killing ability and other immune functions during clinical and subclinical mastitis (Oldham *et al.*, 1991 and Hogan *et al.*, 1992).

Concluding the most common bacteria isolated from goat's milk under this study coagulase negative staphylococci (CNS) appeared to be the most prevalent pathogen group that supported by many authors who accounted CNS for 58 and 93% of the bacteria that cause IMI in goats across various countries and geographical zones (Contreras *et al.*, 2007 and Raynal-Ljutovac *et al.*, 2007). Additionally, staphylococci were the pathogens that most commonly isolated (28.8%) from the mammary glands of goats (Haenlein, 2002 and Leitner *et al.*, 2007). Presently Roukbi *et al.* (2015) found that IMI in goats caused by staphylococcus (75%), *E. coli* alone (16.6%) or associated with streptococci (8.3%).

Furthermore the present results declared that Vit C supplementation not affected the type of bacteria in

both groups, but affected their infection rates represented by their lower percentages and significantly lower TBC in Vit C supplemented group if compared with the milk samples of the control does along the experiment period. This may be attributed to OS led to reduce resistance to the invasion of pathogenic micro-organisms and the probability of appearance of inflammatory conditions especially mastitis increased (Jóźwik *et al.*, 2012a).

Conclusively, the dairy goat (as a model of dairy animals) seemed to have more OS and low antioxidant defense during early lactation or just after parturition than other pregnant goats, and this seemed to be the probable reason for their increased susceptibility to diseases (e.g. mastitis, metritis, retention of fetal membranes etc.) and other health problems. Therefore dietary supplementation with Vit C in this critical period may improve some of their antioxidant defense activities especially on reproductive performance and milk quality.

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العلاقة بين حالة الأكسدة و معدل الحمل وجودة الحليب في الماعز الحلابه المضاف إلى عليقتها فيتامين ج

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أجريت هذه التجربة لدراسة تأثير إضافة فيتامين "ج" كمضاد للأكسدة لتحسين أداء الماعز الحلابه المعرضة لإجهاد الأيض أثناء الفترة الانتقالية (الشهر الأخير من الحمل والمرحلة الأولى من الحليب) وتأثير ذلك على معدل الإخصاب ومستوى كل من مضادات الأكسدة والأجسام الشاردة وهرمون الكورتيزون وهرمونات الغدة الدرقية ومعدل نمو ووزن الفطام للحملان الرضيعة ومستوى جودة ونوعية اللبن المنتج. لهذا الغرض تم استخدام عدد ١٦ من إناث الماعز العجمي في الفترة الأخيرة من الحمل وقد تم تقسيمهم عشوائياً إلى مجموعتين متساويتين، المجموعة الأولى ضابطة: تتغذى على العليقة الأساسية للمزرعة أما في المجموعة الثانية فقد تم إضافة ٧٥ ميللجرام من فيتامين "ج" لكل كيلوجرام من وزن الأنتى للعليقة الأساسية يومياً لمدة ثلاث شهور، شهر قبل الميعاد المتوقع للولادة وشهرين بعد الولادة. تم تجميع عينات الدم مره كل أسبوعين من بداية التجربة حتى نهايتها. أما عينات اللبن فقد تم تجميعها مرة كل ١٥ يوم بداية من أسبوعين حتى شهرين بعد الولادة. أوضحت النتائج وجود زيادة معنويه في مجموعة الماعز المضاف لعليقتها فيتامين "ج" عن المجموعة الضابطة في معدل الخصوبة بعد الولادة ومعدل نمو ووزن الفطام لحملانهم وزيادة نسبة الجلوتاثيون ونقص مستوى الأجسام الشاردة في الدم إلى جانب وجود اختلافات معنويه في نشاط الغدة الدرقية ومستوى الكورتيزون في الدم. بالنسبة لتأثير إضافة فيتامين "ج" لعلائق الماعز الحلاب على جودة ونوعية اللبن المنتج فقد وجد أن إضافة فيتامين "ج" قام بتحسين مستوى معظم مكونات اللبن معنوياً خاصة نسب كل من الدهون واللاكتوز ومستوى اليوريا ومجموع المواد الصلبة و المواد الصلبة الغير دهنيه. في الوقت نفسه كانت هناك زيادة غير معنويه في نسبة بروتين اللبن خلال فترات التجربة المختلفة. علاوةً على ذلك، كان هناك انخفاض معنوي كبير في كل من عدد الخلايا الجسيمية و العدد الكلي للبكتيريا ممثلاً في معدلات الإصابة بالبكتيريا المختلفة وذلك في المجموعة التي تم إعطاؤها فيتامين "ج" مقارنة بالمجموعة الضابطة. وقد خلصت هذه الدراسة إلى أن إضافة فيتامين "ج" إلى علائق إناث الماعز الحلاب في الفترة الانتقالية والتي يكون فيها الحيوان تحت تأثير الإجهاد الأيضي (الفترة الأخيرة من الحمل والمرحلة الأولى من الحليب) قد أدى إلى تحسين الآثار السلبية للإجهاد الأيضي في هذه المرحلة وقد انعكس ذلك على تحسين جودة اللبن المنتج وكذلك زيادة معدل الإخصاب لهذه الماعز بعد الولادة وأيضاً أدى إلى زيادة معدل النمو وكذلك تعزيز وزن الفطام لحملانهم. لذلك توصى هذه الدراسة بإضافة فيتامين "ج" لعلائق إناث الماعز الحلاب في الفترة الانتقالية للتغلب على الآثار السلبية الضارة للإجهاد الأيضي على الوظائف الحيوية المختلفة وتحسينها.