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Impact of hormonal and non-hormonal treatments on milk production and reproductive performance of lactating New Zeland White rabbits

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Abstract

The present study aimed to determine the influence of equine chorionic gonadotropin (eCG), L-Carnitine (LC) and Royal jelly (RJ) treatments on milk production and subsequent reproductive performance of lactating NZW rabbits. A total 28 primiparous lactating New Zeland white (NZW) rabbits divided randomly into four groups (7 animals/ group): Group 1 (eCG group) on day 9 postpartum the females were treated with 50 IU eCG IM/ animal at 48^{hrs} before mating. Group 2 (LC group): the rabbits were given 50 mg LC /kg. BW/ day for 21 days orally starting at 24^{hrs} of parturition. Group 3 (RJ group) before mating the rabbits were given 100 mg RJ/ kg. BW/ day for 21 days orally starting at 24^{hrs} of parturition. Group 4 was Control group (without any treatment). The reproductive parameters including receptivity, conception and kindling rate as well as litter size and weight at weaning (LSW & LWW) and pre-weaning mortality (PWM) were recorded. The results revealed that there were no significant differences in the number of services per conception, conception and kindling rate among eCG, LC, RJ and control groups. Milk production, LSW and PWM were significantly improved after LC or RJ compared to eCG and control. In conclusion, the non-hormonal treatment (LC or RJ) supplementation can improve milk production and this positively reflected on LSW, LWW and PWM.

Keywords: eCG, L-Carnitine, royal jelly, primiparous rabbits, milk production.

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1. Introduction

During the postpartum period, approximately 60% of lactating primiparous does are infertile and must highlighting on the negative effect of lactation on reproduction in such period (Lange and Schlolaut, 1989; Rebollar *et al*., 1992). In rabbit farms the most widely used hormone in lactating non-receptive does is eCG (Rebollar *et al*., 2006), eCG injection two or three days before insemination has become very common in industrial management as this practice generally improves reproductive performance and it is simple to use (Gogol, 2004). Concerning of milk production after treatment with eCG, several works have consistently found that milk production is reduced in pregnant lactating rabbits versus lactating only (Hudson *et al*., 1996). LC can promote fatty acid and energy application by transporting long chain fatty acids across the inner mitochondria membrane for β-oxidation, thus enhance adenosine triphosphate (ATP) concentration (Vanella *et al.*, 2000). Seleem *et al*. (2006) observed that the increase milk secretion and its yield in treated rabbits with LC as well as litter size and weight at birth significantly higher and pre-weaning mortality rate significantly lower than those of drank free LC water. Royal jelly (RJ) consists of a milky fluid secretion produced by the pharyngeal glands of bees 5 to 10 days old and has the primary function of feeding the queen, producing an individual anatomically and physiologically different from the workers, with development of reproductive capacity (Kohno *et al*., 2004). It has a complex composition water, proteins, lipids, carbohydrates, amino acids, mineral salts, vitamins, enzymes, hormones and natural antibiotics (Mărghitaş, 2008). Gimenez-Diaz *et al*. (2012) suggested that a single RJ (500 mg) injection increased overall reproductive performance of seasonal sheep breeds in the anestrus season. Also, Bonomi and Bonomi (2004) found that the addition of RJ was able to induce a significant improvement of milk yield, milk fat, and milk total protein and improved reproductive efficiency in dairy cattle. The aim of the present study was to investigate the influence of hormonal treatment (eCG) and non-hormonal treatments (RJ or LC) on milk production and subsequent reproductive performance of lactating NZW rabbits.

2. Materials and methods

2.1 Animals and Management

The present study was carried out at private farm in Mallawy, El-Minia government, Egypt (located at latitude of 28° 07' 6.00" N, longitude of 30° 44' 23.99" E and about 52 meters above sea level). The study was conducted on a total 28 primiparous lactating NZW rabbits aged between 5.5 - 6 months and with average weight of (2936.25±19.54 gm). All rabbits were kept individually in suspended galvanized wire cages, provided with feeders and drinkers, receiving water, commercial feed (ALMARAI, 18% protein).

2.2 Experimental design and treatments

The rabbits were divided randomly and equally into four experimental groups (7 animals / group) as follow: Group 1 (eCG group), on day 9 postpartum, the lactating females were treated with 50 IU eCG (Gonaser, Hipra, Girona Spain) IM/animal at 48^{hrs} before the time of natural mating. Group 2 (L-Carnitine group), (Mepaco, Egypt) before mating, the rabbits were given 50 mg LC/ kg. BW/day for 21 days orally starting at 24^{hrs} of parturition. Group 3 (Royal Jelly group), Egyptian fresh RJ was obtained from a local bee products company (KONOZ Co., Egypt), before mating, the rabbits were given 100 mg RJ/kg. BW/day for 21 days orally starting at 24^{hrs} of parturition. Group 4 (Control group), left without treatment and mated after day 21 of lactation. Milk yield was estimated after deprivation of the pups from suckling their mothers for 24^{hrs} according to Hudson and Distel, (1982), when the kits started to consume solid feed, from day 18 of lactation onwards, the kits housed in a separated cage to allow both the milk yield determination as well as the normal development of the kits according to Fortun-Lamothe *et al*. (2000), the pups were weighted before and after suckling, the increase in pups weight was used as the doe milk yield (Seleem *et al*., 2006). In the all previous groups (G1, G2, G3 and G4) natural mating by fertile bucks was done. At kindling, litter size and weight at birth were recorded. Litter size and weight at weaning ((LSW, LWW, 28 days of age) as well as Pre weaning mortality (PWM) were recorded per doe.

2.3 The items were recorded for each treatment group

- Sexual receptivity rate, conception, number services per conception, kindling rate and litter size at birth.
- Milk yield per doe (total and daily /gm).
- Litter size and weight at 28 days of lactation (weaning) and Pre weaning mortality per doe were recorded.

2.4 Statistical analysis

All data were analyzed by Statistical Package for the Social Sciences (SPSS, version 20) software and presented as mean ± SE. Significant differences among the groups (number services/conception, milk production, LSB, LSW, LWW, PWM and biochemical parameters) was performed by one-way ANOVA followed by post-hoc Duncan test (Duncan, 1955). Receptivity, conception and kindling rate were compared between treatment groups using the chi-square test. The statistical significant differences were accepted at ($P<0.05$).

3. Results and Discussion

3.1 Reproductive performance of lactating New Zeland white rabbits

The present results showed that there were no significant differences of receptivity, number services per conception, conception and kindling rate among eCG, LC, RJ and control groups (Table 1). The results regarding to eCG effects on reproductive activity of lactating rabbits in coincided with Quintela *et al*. (2001) where they found that no significant differences of eCG treatment on receptivity or kindling rate in lactating rabbits. Also, Theau-Cle´ment *et al*. (2008) showed that no significant effect of this hormone (eCG) in the fertility of primiparous does. To our knowledge there are no available studies at yet about the effect of LC or RJ treatment on the receptivity, conception and kindling rates in lactating rabbits, however, the obtained results indicated that the application of LC or RJ to rabbits during lactation had not negative effects on reproductive activity and tended to have higher receptivity and conception rate addition to decrease services index compared to untreated does.

Table (1): Effect of hormonal (eCG) and non-hormonal (LC, RJ) treatments on reproductive performance of lactating primiparous New Zeland white rabbits (n=7; Mean±SM).

Groups	Reproductive performance				
	Receptivity % (n)	NO Services/ conception	Conception % (n)	K Kindling % (n)	LSB
eCG	85.71(6/7)	1.25±0.25	66.67(4/6)	100(4/4)	7.00±0.40[a]
LC	85.71(6/7)	1.20±0.20	83.33(5/6)	100(5/5)	6.80±0.37[a]
RJ	85.71(6/7)	1.50±0.28	66.67(4/6)	100(4/4)	5.50±0.28[b]
Control	71.43(5/7)	1.66±0.33	60.00(3/5)	100(3/3)	5.00±0.57[b]
Sig	NS	NS	NS	NS	0.01

Different superscript letters indicate significance within the same column (p<0.01). NS = No Significant. LSB = Litter Size at Birth.

3.2 Litter size at birth (after lactation)

The results showed that, litter size at birth (LSB) in eCG and LC groups significantly higher than that in RJ and control groups, were (7.00±0.40 and 6.80±0.37 VS 5.50±0.28 and 5.00±0.57, respectively; P<0.01) (Table 1). Increase of the litter size at birth after LC treatment in lactating rabbits may be due to enhancement of ovarian follicles prior mating as well as increase ovulation rate after mating. Litter size is determined by ovulation rate, fertilization and/or prenatal survival in rabbits (Blasco *et al*., 1993; Belabbas *et al.,* 2016). The current results agreed with the finding by Rebollar *et al*. (2006) who recorded that eCG treatment increased prolificacy (more kits born) in lactating does compared to controls. Also, Bonanno *et al*. (1990) showed that eCG provoked significant follicular growth, which could explain a higher ovulation rate and more litters at birth. Many studies observed that the application of LC prior mating improved follicular growth in mice (Miyamoto *et al*., 2010) and ewes (El-Shahat and Abo-Elmaaty, 2010) and increased ovulation rate in rabbits (Abdel-Khalek *et al*., 2016).

3.3 Milk production

Total milk yield during lactation period increased significantly (p<0.01) in LC VS RJ and was higher than that in control and eCG groups (2950.00±79.07 VS 2636.80±40.28 VS 2297.16±67.21 and 2172.66±129.65/gm/doe, respectively) (Table 2). Likewise, the daily milk yield increased significantly ($p<0.01$) in LC VS RJ and higher than that in control and eCG groups (105.35±2.82 VS 94.17±1.43 VS 82.04±2.40 and 77.59±4.63/gm/doe, respectively). Increase milk yield (total and daily) in rabbits treated with LC may be due to the positive activities of LC as antioxidant on balance of energy, metabolic and mammary glands health during lactation. Eder *et al*. (2004) observed that sows supplemented with LC had larger and more active mammary glands than control animals. Furthermore, Scholz *et al*. (2014) recorded that LC improved metabolic health during lactation and showed a trend of improving milk production in dairy cows. The present results coincided with those reported by Seleem *et al*. (2006), where they observed increase milk secretion yield in treated rabbits with L-Carnitine. Furthermore, Pirestani and Aghakhani (2018) found that, LC had favorable effect on milk production; this can be attributed to the positive effects of LC on reducing the negative balance of energy and protein production. There are no available studies at yet about the effect of RJ treatment on milk production in rabbits, the increase in milk yield after RJ treatment may be due to the complex components found in RJ as a source of nutrients which might be involved in metabolic pathways and tissue synthesis, RJ have vasodilative activities, have antioxidant effects and increase energy production. It reported that RJ has been demonstrated to possess several pharmacological activities in experimental animals, as metabolic and antioxidant effects (Liu *et al*., 2008) and have vasodilative activities (Shinoda *et al*., 1978) which might increase blood flow to mammary glands and improvement its functions. A number of workers outlined the effects of RJ treatments in other species. Bonomi and Bonomi (2004) found that the addition of royal jelly was able to induce a significant improvement of milk yield in dairy cattle. Also, El-Tarabany *et al*. (2019) recorded that the ewes supplemented with RJ had higher in daily milk yield than untreated ewes.

Table 2: Effect of hormonal (eCG) and non-hormonal (LC, RJ) treatments on milk production, LSW, LWW and PWM of lactating NZW rabbits (Mean±SEM).

Groups	Total milk yield /gm	Daily milk yield /gm	LSW	LWW	PWM
eCG	2172.66±129.65^{c}	77.59±4.63^{c}	3.57±0.29^{b}	372.41±16.26^{c}	2.71±0.28^{a}
LC	2950.00±79.07^{a}	105.35±2.82^{a}	5.57±0.42^{a}	428.94±11.84a^{b}	0.71±0.28^{b}
RJ	2636.80±40.28^{b}	94.17±1.43^{b}	5.28±0.35^{a}	463.18±17.09^{a}	1.14±0.34^{b}
Control	2297.16±67.21^{c}	82.04±2.40^{c}	4.14±0.26^{b}	386.47±24.07^{bc}	2.71±0.42^{a}
Sig	0.01	0.01	0.01	0.01	0.01

Different superscript letters indicate significance within the same column ($p<0.01$). LSW = Litter Size at Weaning. LWW = Litter Weight at Weaning (Mean individual). PWM = Pre Weaning Mortality.

The results regarding to eCG effect on milk production in coincide with Rebollar *et al*. (2008) reported that the eCG when administration at day 9 postpartum lead to decrease in PRL concentrations. The rabbits treated with eCG at day 9 postpartum (day 9 of lactation) showed lower in milk production compared to other group, this decreased may be due to concurrent of lactation with pregnancy. This group treated with eCG at day 9 postpartum and mated at day 11 postpartum and the pregnancy was done (four does came pregnant from total group; Table 1) during lactation period. The decline in milk yield due to the gestation overlapping is a result of the pregnancy requirements that consistently increase with the exponential fetal development (Parigi-Bini and Xiccato, 1998).

3.4 Litter size and weight at weaning and preweaning mortality

At the end of lactation period (28 days) Litter size and weight at weaning (LSW, LWW) and preweaning mortality (PWM) were recorded and showed in Table (2). LSW was significantly ($p<0.01$) higher in LC and RJ groups than that in control and eCG groups (5.57±0.42 and 5.28±0.35 VS 4.14±0.26 and 3.57±0.29, respectively). LWW was significantly ($p<0.01$) higher in RJ than in control and eCG groups (463.18±17.09 VS 386.47±24.07 and 372.41±16.26/gm, respectively), but no significant difference compared to LC group (428.94±11.84/gm) (Table 2). Pre-weaning mortality (PWM) was significantly ($p<0.01$) lower in LC and RJ groups than in eCG and control groups (0.71±0.28 and 1.14±0.34 VS 2.71±0.28 and 2.71±0.42/doe, respectively; Table 2). This mean there was improvement in the fetal viability in both LC and RJ groups. This improvement in LSW, LWW and PWM in LC and RJ groups may be attributed to the high milk production resulting of these treatments (as showed in Table 2). There was a positive correlation between litter size and milk yield in rabbits (Lebas *et al*., 1997; Rommers *et al*., 2001), growth and survival of rabbit kits depend exclusively on the doe's milk production as a first food for the newly born offspring (Kapadiya *et al*., 2016). The results recorded by Jimoh and Ewuola (2017) indicated that the high milk yield of rabbit does increased survival rate and average litter weight at weaning. Also, Khalil (1980) acknowledged that decline in mortality due to improvement in maternal care through the does' capacity to produce adequate milk and suckle her young. The results regarding to the positive effect of LC in agreement with those reported by Seleem *et al*. (2006), where they found that the pre-weaning mortality significantly lower in rabbit does supplemented with LC than control. Furthermore, Ramanau *et al*. (2004) found that the piglets of sows supplemented with LC grew faster during the suckling period than piglets of control sows; this suggests that this effect might be due to a higher milk yield and an increased transfer of energy and nutrients from the sow to the piglets with the milk. Addition to the positive effect of RJ on milk production, increase litter size and weight at weaning and decrease PWM in lactating rabbits treated with RJ may be also due to the positive effect of RJ on milk composition. Early live ability and growth performances of newborn rabbits are closely related to the quantity and quality of the milk ingested (Szendrö and Maertens, 2001). El-Tarabany *et al*.

(2019) recorded that the supplementation of RJ improves daily milk yield and the contents of milk (proteins, fats and total solids) in lactating Ossimi ewes. There were not improve in LSW, LWW and PWM of lactating rabbits treated with eCG, this may be due to the negative effect of eCG on milk production (Table 2). These results in agreement with those reported by Maertens and Luzi (1995), where they observed that eCG does not improve the litter size and weight at weaning. Furthermore, Mehaisen and Abbas (2014) found that the number of weaned kits significantly decreased in eCG group (3.8 kits) in comparison with control group (6.1 kits).

4. Conclusions

In conclusion, the non-hormonal treatment (LC or RJ) supplementation can improve milk production and this positively reflected on LSW, LWW and PWM, as well as LC or RJ hadn't negative effect on reproductive performance of primiparous NZW rabbits. The application of eCG at day 9 postpartum (semi-intensive rhythm) causes decline milk production and this negatively reflected on LSW, LWW and PWM.

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