IMPACT OF DIETARY SUPPLEMENTATION OF MILK THISTLE (*Silybum marianum*) SEED EXTRACT ON DOE RABBITS PERFORMANCE

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SUMMARY

Milk thistle seed extract consists mainly of flavonolignans, which include silybin (silibinin), sylichrisitn, and silydianin that commonly referred to Silymarin. A total number of thirty six, 9-10 month old New-Zealand white muliparous does, with an average weight of 3641g ± 94.02 were used to evaluate the response to dietary supplementation with ethanolic milk thistle extract (MTE) containing 70% silymarin on some performance aspects during pregnancy and lactation periods. Does were allocated to 4 experimental groups, 9 does of each. Rabbits were fed the experimental diets started one month before first mating. Does were allowed to the following treatments; a control group was fed a basal diet without MTE (T1), then the 2nd, 3rd, and 4th were fed the basal diet supplemented with 3ml, 6ml or 9ml MTE/kg diet, respectively and abbreviated as 3MTE (T2), 6MTE (T3) and 9MTE (T4), respectively. The trail was ended after pregnant period and nursering kits till weaning. The results indicate that feed intake during pregnancy (except T2) and lactation , litter weight at birth and weaning (except T2) were significantly (P≤0.05) increased by supplementing does diets with MTE at 6 or 9ml/kg diet compared to the control. Also, litter size at birth and litter size at weaning (except T2) were significantly (P≤0.05) higher with MTE supplementation compared to the control. Milk production significantly (P≤0.05) was the highest in 9MTE group for the first four weeks compared with the control. Plasma prolactin hormone was significantly (P≤0.05) improved gradually by enriched does diet with MTE, but liver enyzmes (AST & ALT) was insignificantly higher in all MTE groups compared to the control. It could be concluded that adding milk thistle seed extract to does diets enhanced their performance during gestation and lactation periods, in term of increased their milk production and litter weight at birth and weaning.

***Keywords**: Milk thistle extract, doe rabbits, milk production and prolactin.*

INTRODUCTION

Rabbit does are in general allowed to nurse their kits till weaning age (4-5 weeks of age). Kits until 18-19 days of age exclusively depending on the milk of their mother (Fortun-Lamothe *et al.*, 2000). Newborn rabbits have high energy requirements and a low thermal isolation. Therefore early livability and growth performances are closely related to the quantity and quality of the milk ingested (Szendrö and Maertens, 2001). Consequently, demands and requirements of does for milk yield have increased greatly. However, strains were primarily successfully selected for increased litter size with lower weight of kits (Rochambeau, 1998). This indicates that the relative increase of milk yield was smaller than that of litter size, leading to smaller amounts of milk available per kit (Szendrö and Maertens, 2001).

Studies have shown that milk yield of doe rabbits are strongly influenced by diet composition (Maertens *et al.*, 2006 and Pascual *et al.*, 2002). Milk thistle (*Silybum marianum* L.) contains silymarin which is a mixture of flavonolignans, mainly silibinin (also known as silybin). It has been widely studied for its hepatoprotective (Giese, 2001), anti-inflammatory (Gupta *et al.*, 2000), antioxidant and choleric properties (Nencini *et al.*, 2007), but silymarin is also used in alternative medicine to increase milk yield

in women with hypogalactia (Di Pierro *et al.,* 2008). Such a beneficial effect was also seen in domestic species, as silymarin increased milk production in cows (Tedesco *et al.,* 2004). Silymarin is a standardized preparation extracted from the fruits (seeds) of milk thistle. Milk thistle is a purported galactogogue (Jackson,2010). Some studies show that herbal galactagogues can increase breast milk production (Forinash *et al.*,2012, Zapantis *et al.* 2012 and Mortel and Mehta 2013). Studies using animal models showed that extracts of herbal galactagogues, such as Silitidil which is a standardized extract of milk thistle and Silymarin BIO-C which is an extract from *Silybum marianum* fruits, increased serum prolactin levels significantly in female rats and that this appears to be the mode by which milk production is increased (Capasso *et al.* 2009, and Capasso 2014). This study aims to evaluate the effect of graded levels of milk thistle seed extract on performance of pregnant and lactating doe rabbits and their off-springup to weaning.

MATERIALS AND METHODS

The experimental work was carried out at Sakha Animal Production Research Station, Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture, Dokki, Giza, Egypt.

Preparations of milk thistle extract (MTE):

A sample of 500 g of *Silybum marianum* seed powder was placed in a thimble made of filter paper and inserted into the wide central tube of the extractor of the Soxhlet apparatus. The solvent (ethanol 95%) was placed in the flask and heated at 78 °C and their vapor was condensed in a reflux condenser. The condensed extract was dripped into the thimble containing the crude component, which was extracted by contact. When the level of the liquid in the chamber had risen to the top of the siphon tube, the liquid contents of the chamber was drained off into the flask. This process was continued until a drop of the solvent from the siphon tube did not leave any residue when evaporated (Handa, 2008). The extract was stored in a refrigerator (4 °C) until used and was mixed with feed before pelleting. Active component of the extract was analyzed according to Cai *et al.* (2009), which contains approximately 70% determined silymarin.

Experimental animals, design and management:

Thirty six multi-parious New Zealand White does 9-10 months old weighing 3641g ±94.02 were equally allocated to four groups as follows:

T1 (control): fed basal diet without supplementation.
T2: fed basal diet + 3 ml MTE/kg diet (3MTE).
T3: fed basal diet + 6 ml MTE/kg diet (6MTE).
T4: fed basal diet + 9 ml MTE/kg diet (9MTE).

Each group had 9 does, which were fed the experimental diets for one month before first mating. Detection of conception was carried out by palpation at 10 days after mating and the non pregnant were re-mated immediately. Does were housed in individual wired-cages. All animals were kept under the same management and hygienic conditions and provided with fresh water and pelleted diets *ad-libtum* over the experimental period.

Experimental diets and measurements*:*

Diets were formulated to meet the Agriculture ministry decree (1996) requirements during pregnancy and lactation periods. Ingredients and calculated analyses of the basal diet are presented in Table (1). Variables of does weight, doe feed intake, litter size and weight at birth and at weaning were considred. Individual blood samples were collected from three does of each treatment from the marginal ear vein in 5 ml heparinized test tubes and centrifuged at 3000 r.p.m for 20 minutes then plasma were stored at -20ºC until analysis for aspartate amino-transferase, AST, alanine amino-transferase, ALT (Biosystems, S.A. , Spain) according to Reitman and Frankel (1957) and prolactin levels (ELISA kit (ABO Swiss Co., Ltd, China) according to Kletzky *et al.* (1980).

Table (1): Ingredients and calculated analysis of the basal diet.

Ingredient	%	Calculated chemical analysis	
Barley	17.5	Crude protein (%)	18.26
Wheat bran	32.2	Digestible energy (kcal/kg)	2620
Soybean meal (44%)	13.6	Crude fiber (%)	11.14
Clover hay (12%)	17.9	Ether extract (%)	2.75
Corn gluten (60%)	3.5	Calcium (%)	1.23
Yellow corn	11.5	Total phosphorus (%)	0.81
Limestone	1.5	Lysine (%)	0.82
di-Calcium phosphate	1.5	Methionine (%)	0.51
NaCl	0.3	Methionine + cystein	0.84
Vitamins and menial premix*	0.3	Sodium	0.16
Dl- Methionine	0.2		
Total	100		

**Each 3 kg contain: 6000000 IU Vit. A; 900000 IU Vit. D3; 40000 mg Vit. E; 2000 mg Vit. K3; 2000 mg Vit. B1; 4000 mg Vit. B2; 2000 mg Vit. B6; 10 mg Vit. B12; 50 mg Biotin; 10000 mg Pantothenic acid; 50000 Niacin; 3000 mg Folic acid; 250000 mg Choline; 8500 mg Mn; 50000 mg Zn; 50000 mg Fe; 200 mg I; 100 mg Se, 5000 mg Cu, and 100 mg Co.*

Milk production and composition:

Milk production was estimated by using doe-suckle-weigh method (Lukefahr *et al.*, 1983). Milk samples were drawn on d 21after parturition. Milk composition was evaluated for total lipid, total protein, and albumin content by infrared analyzer with a Milkoscan (Milk-o-Scan 605, Foss Electric, Hillerd, Denmark) according to the method of El-Sayiad *et al.* (1994). While, globulin was calculated by subtracting albumin from total protein and A/G ratio was also calculated.

Statistical analysis:

The obtained data were statistically analyzed using one-way analysis of variance procedure (SAS, 2001) computer program using the following model:

$$Y_{ij} = \mu + T_i + e_{ij},$$

Where Y_{ij}= the individual observation. μ = overall mean, T_i = effect of treatments (i = 1, 2, 3 and 4). e_{ij} = random error.

Significant differences between treatments means were determined at ($P \leq 0.05$) and ($P \leq 0.01$) by Duncans multiple-range test (Duncan's, 1955).

RESULTS AND DISCUSSION

Results in Table (2) show that the highest pregnant does feed intake was observed in T4 (fed 9MTE) without significant difference to those of T3 (fed 6MTE), while the lowest consumption of feed was recorded for T2 (fed 3MTE) without significant differences to control (T1). This finding may be due to improve the palatability of the diet by increasing MTE level. These results disagree with the findings of Attia *et al.* (2017) who concluded that feeding rabbit bucks with dietary ground milk thistle seeds reduced their feed intake compared to the control group. All of the tested groups recorded significantly higher litter size by 15.4%, 28.2% and 20.5%, respectively compared to control group. The improvement in these groups might be due to improving the ovulation rate in does fed MTE compared to control as reported by Taher *et al.* (2012) who found that treated women with silymarin (750mg/day) enhanced their ovulation rate by incrementing progesterone hormone levels. Group of 6MTE and 9MTE achieved significantly higher litter weight compared to control and 3MTE groups. These results confirm the latest research of Mohammad *et al.* (2019) who documented that feeding female rat with 200 mg\kg\day of milk thistle extract resulted in higher litter weight at birth compared to control.

Table (2): Effect of different levels of milk thistle seeds extract on does performance during gestation period.

Item	T1	T2	T3	T4	±SE	Sig.
Mating weight (g)	3756	3694	3641	3699	94.02	NS
Feed intake (g)	4264 [bc]	4193 [c]	4824 [ab]	5086 [a]	193	*
Live litter size at birth	5.57[b]	6.43 [a]	7.14 [a]	6.71 [a]	0.24	*
Litter weight at birth (g)	262.14 [a]	271.43 [b]	326.43 [a]	311.43 [a]	12.04	*

[a], [b] and [c] Means within the same column with different superscripts are significantly different,
** P≤0.05, NS= non significant.*
T1 (control), T2: fed basal diet + 3 ml MTE (3MTE),
T3: fed basal diet + 6 ml MTE (6MTE), T4: fed basal diet + 9 ml MTE (9MTE).

According to milk production (Table 3), rabbits of T4 fed the highest level of MTE (9 ml) T4 group recorded significantly the best milk yield during the first 4 weeks after delivering bunnies, while at last week of lactation (fifth week) this improvement was disappeared. These results are in agreement with Mohammad *et al.* (2019) who reported that silymarin increased the activity of mammary gland and thus milk production in female rats. There are several reviews speculated that milk thistle seed stimulate milk production as tested in rats (Capasso *et al.*, 2009) and dairy cow (Tedesco *et al.*, 2004). This observation has gained a renewed interest in its galactogenic properties. Moreover, Silymarin reduced the negative conditions experienced in transition to metabolic adaptation at the beginning of lactation and thus improved the milk yields (Ulger *et al.*, 2017).

Table (3): Effect of different levels of milk thistle seeds extract on does milk production during different weeks.

	T1	T2	T3	T4		
Item	Average milk production (ml/doe/day)				±SE	Sig.
Week 1	43.3 [c]	64.2 [b]	68.6 [b]	94.3 [a]	5.94	**
Week 2	95.8 [b]	93.3 [b]	131.7 [ab]	149.3 [a]	13.6	*
Week 3	125.8 [c]	131.0 [c]	177.1 [b]	249.3 [a]	14.7	**
Week 4	127.1 [b]	160.8 [ab]	131.7 [b]	192.9 [a]	16.5	*
Week 5	141.7	122.0	122.5	131.7	13.4	NS

[a], [b] and [c] Means within the same column with different superscripts are significantly different ,
** P≤0.05, **P≤0.01, NS= non significant.*
T1 (control), T2: fed basal diet + 3 ml MTE (3MTE),
T3: fed basal diet + 6 ml MTE (6MTE), T4: fed basal diet + 9 ml MTE (9MTE).

During lactation period (Table 4), it is worthy to note that by increasing the level of MT in does diet, feed intake was gradually (P≤0.05) increased compared to control. does fed either 6MTE (T3) or 9MTE (T4) recorded significantly higher weaning litter size by 41.2% and 29.4%, respectively compared to control. This confirms the result of increasing milk yield in these groups. Moreover, silymarin (the active component of MTE) has antioxidant effect according to Kshirsagar *et al.* (2013) what cause a protective action against lipid oxidation in the cell membrane (Liebler, 1992). Also, it is important for newborns which exhibits a greater sensitivity to oxidative damage than adults, and for the development of the immune system in young animals (Debier *et al.*, 2005). By increasing dietary MTE level from 6ml to 9ml in does diet, the weaning litter weight (5 weeks) was increased significantly by 15.5% and 28.8%, respectively compared to untreated group. The improvement in weaning weight of bunnies may be due to increasing in milk yield as reported previously in Table (3). These results agree with Mohammad *et al.* (2019) who concluded that feeding silymarin in female rats improved significantly their litter weight as a result of increasing milk production.

Table (4): Effect of different levels of milk thistle seeds extract on does performance during lactation period.

Item	T1	T2	T3	T4	±SE	Sig.
Feed intake (g)	5757 [b]	6820 [a]	6927 [a]	7043 [a]	23.7	*
Live litter size at weaning	4.86 [c]	5.57 [bc]	6.86 [a]	6.29 [ab]	0.34	**
Litter weight at weaning (g)	2177 [c]	2198 [c]	2514 [b]	2804 [a]	90.14	**

[a], [b] and [c] Means within the same column with different superscripts are significantly different ,
** P≤0.05, **P≤0.01.*
T1 (control), T2: fed basal diet + 3 ml MTE (3MTE),
T3: fed basal diet + 6 ml MTE (6MTE), T4: fed basal diet + 9 ml MTE (9MTE).

Although there is a gradual increase in serum AST and ALT values in does fed gradual level of MTE, the differences were none significant and values were within normal rage (Table 5). In this connection, Ulger *et al.* (2017) found that adding 20 g silymarin/ head / day for Holstein cow did not affect AST and ALT activity. According to prolactin level in serum, does fed diet enriched with MTE at 3 and 6 ml/kg diet recorded the same improvement (P≤0.05) in prolactin value by 13.5% compared to control. Moreover, does fed 9MTE diet achieved significantly the highest value by 19.0% (Table 5). This improvement in prolactin value in tested groups may be due to anti-dopaminergic activity (Capasso, 2014) and the estrogenic effects (Demirci *et al.*, 2014) of silymarin. In this respect, Capasso *et al.* (2009) and Capasso (2014) found that female rats administered with extracts of herbal galactagogues, such as Silitidil which is a standardized extract of milk thistle and Silymarin BIO-C (an extract from Silybum marianum fruits), increased serum prolactin levels significantly. Pituitary gland activate the secretion of prolactin hormone which in term increases two functions of the mammary glands such as the growth during pregnancy then stimulate of milk production during nursing infants (Ben-Jonathan *et al.*, 2006). Recently, Mohammad *et al.* (2019) concluded that silymarin (which has galactogenic properties), increased serum prolactin levels in female rats during pregnancy and lactation.

Table (5): Effect of different levels of milk thistle seeds extract on does blood plasma constitutes.

Item	T1	T2	T3	T4	±SE	Sig.
AST (U/ml)	27.22	29.11	39.82	40.95	6.60	NS
ALT (U/ml)	26.69	32.86	35.89	38.12	6.40	NS
Prolactin (ng/ml)	2.00[b]	2.27 [a]	2.27 [a]	2.38 [a]	0.07	**

[a], and [b] Means within the same column with different superscripts are significantly different ,
***P≤0.01, NS= non significant.*
T1 (control), T2: fed basal diet + 3 ml MTE (3MTE),
T3: fed basal diet + 6 ml MTE (6MTE), T4: fed basal diet + 9 ml MTE (9MTE).

Regarding to milk composition (Table 6), doe rabbits fed either control or 9MTE diets recorded higher total protein and albumin values than others fed 3MTE (T2) and 6 MTE (T3), while, globulin and A/G ratio values were not affected significantly by any of the tested diets. These results disagree with the finding of Ulger *et al.* (2017) who concluded that treated Holstein dairy cattle with 20 g silymarin/head/day, had a reduction in milk protein. Total lipids in milk were significantly higher in group fed 9MTE followed by control and 3MTE. However, the least value was recorded with 6MTE group. This finding may be due to that silymarin did not have a cholagogue effect (Saeed *et al,.* 2017) which mean that it is an agent to promote higher flow of bile and it reflect on high milk lipids. The current results conflict with previous research of Tedesco *et al.* (2004) who found that dairy cows orally taken 10 g silymarin/day did not show any effect on milk composition (protein and fat%). In another study, Ulger *et al.* (2017) reported that milk fat was significantly decreased by supplementing dairy cattle ration with silymarin.

Table (6): Effect of different levels of milk thistle seeds extract on does milk composition.

Item	T1	T2	T3	T4	±SE	Sig.
Total protein (g/dl)	13.94 [a]	11.32[b]	11.20 [b]	12.50[ab]	0.53	**
Albumin (g/dl)	8.65[a]	6.20[b]	6.63[b]	7.27[ab]	0.47	**
Globulin (g/dl)	5.29	5.12	4.57	5.23	0.21	NS
A/G ratio	1.63	1.23	1.45	1.40	0.10	NS
Total lipids (mg/dl)	1184.2[b]	948.5[bc]	907.3[c]	1581.2[a]	79.63	**

[a, b] and [c] Means within the same column with different superscripts are significantly different ,
***P≤0.01, NS= non significant.*
T1 (control), T2: fed basal diet + 3 ml MTE (3MTE),
T3: fed basal diet + 6 ml MTE (6MTE), T4: fed basal diet + 9 ml MTE (9MTE).

CONCLUSION

It can be concluded that feeding doe rabbits with diets enriched with milk thistle seeds extract has a great affects on milk production (galactogogues effect), and improve litter weight at birth and weaning.

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تأثير اضافة مستخلص حليب الشوك للعلائق على الاداء الانتاجى لامهات الارانب

أميره محمود رفاعى ، مرفت نبيل غزال ، عنايات ابو العزايم و مروى حسنى عبد المجيد

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مستخلص حليب الشوك عبارة عن فلافونوليجنان والذى يتكون من سيليبين وسيليكريستين وسليديانين والتى تعرف بصفة عامة بالسليمارين والذى استخدم فى هذه الدراسة حيث اجريت على عدد 36 أرنب أم سلالة النيوزلندى الابيض (9 – 10 شهور) تزن 3641 جرام ± 94,02 وذلك لتقييم الاستجابة لاضافة مستخلص حليب الشوك (مستخلص اثيرى يحتوى على 70% سليمارين) على الاداء الانتاجى خلال مرحلتى الحمل والرضاعة. تم تقسيم الامهات الى 4 معاملات بكل منها 9 ارانب وتمت تغذيتهم قبل التلقيح بشهرعلى المعاملات الاتية: المعاملة الاولى (كنترول) تغذت على عليقة قاعدية بينما المعاملات الثانية والثالثة والرابعة تغذت على عليقة قاعدية مضاف اليها مستخلص حليب الشوك بمعدلات متدرجة 3 ملليلتر , 6 ملليلتر و 9 ملليلتر /كجم علف على التوالى. وقد استمرت فترة الدراسة خلال فترة الحمل حتى فطام الارانب الصغيرة. وقد اشارت النتائج الى زيادة معنوية فى كلا من الغذاء الماكول خلال الحمل (ماعدا المجموعة المغذاه على 3 ملليلتر/ كجم علف)والرضاعة ووزن الارانب الصغيرة عند الولادة والفطام [فيما عدا المجموعة الثانية (3 ملليلتر/ كجم علف)] عند تغذية الامهات على 6 و 9 ملليلتر /كجم علف مقارنة بمجموعة الكنترول, ايضا زاد معنويا عدد الارانب الصغيرة عند الميلاد والفطام [فيما عدا المجموعة الثانية (المغذاة على 3 ملليلتر /كجم علف)] للمجموعات المغذاه على علائق مضاف اليها مستخلص حليب الشوك مقارنة بمجموعة الكنترول, زاد معنويا معدل انتاج اللبن فى المجموعة المغذاه على 9 ملليلتر /كجم علف فى أول 4 أسابيع من الرضاعة مقارنة بمجموعة الكنترول, تحسن معنويا هرمون البرولاكتين فى بلازما الدم بالزيادة التدريجية لاغناء العلائق بالمستخلص لكن انزيمات الكبد (AST & ALT) زادت بصورة غير معنوية مع اضافة المستخلص مقارنة بالكنترول.

يستخلص من هذه الدراسة ان اضافة مستخلص حليب الشوك لعلائق امهات ارانب النيوزلندى الابيض حسنت من ادائها الانتاجى خلال مرحلتى الحمل والرضاعة ممثلا فى زيادة انتاج اللبن ووزن الارانب الصغيرة عند الميلاد والفطام.