

Soy lecithin in diets for rabbit does improves productive and reproductive performance

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Soy lecithin (SL) is widely used in the food industry as an emulsifier, antioxidant, and wetting agent and due to its composition and properties, it might be used as a nutritional supplement for animals. To this purpose, forty V-line rabbit does were used to estimate the effect of dietary supplementation of SL on productive and reproductive responses during winter and summer seasons. The animals were divided into four groups of 10 does each. The first group served as the control, while the second, third and fourth groups were supplemented with dietary SL at 0.5, 1.0 and 1.5%, respectively. SL supplementation reduced the total content of saturated fatty acids and increased the total unsaturated fatty acid content of doe milk when provided at 1.5% during both seasons ($P<0.05$). When provided at 1 and 1.5% SL inclusion levels, feed intake was reduced ($P<0.05$), while at the 1.5% addition level the body weight of the rabbit does increased after mating and kindling ($P<0.05$). The body weight of rabbit does decreased during summer ($P<0.05$). Both inclusion levels of 1% and 1.5% improved the size and weight of the kits, receptivity, conception rate and the number of service per conception compared to the control group. The SL supplementation \times season interaction affected milk production; 1-1.5% SL increased milk production in both seasons ($P<0.05$), with milk production always being higher during winter compared to summer. The addition of 1.0% soybean lecithin improved productive and reproductive traits of the V-line rabbit does and growth performance of their kits up to weaning in the winter and summer seasons.

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Soybeans are the primary source of vegetable lecithin and soy lecithin (SL), consisting of a mixture of phospholipids, phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol [Loncarevic *et al.* 2013]. The average contents of phosphatidylcholine, phosphatidylethanolamine and inositol phosphatides of SL were reported as 19-21%, 8-20% and 20-21%, respectively [Scholfield 1981], while that of phosphatidylserine as 0.2%–6.3% [Liu and Ma 2011]. Because of its structural and composition properties, soy lecithin is widely used as an emulsifier, antioxidant, wetting agent, and nutritional supplement in animal feed [Li 2006], e.g. for chickens [Siyal *et al.* 2017], dairy cows [de Nardi *et al.* 2012] and fish [Azarm *et al.* 2013].

Thanks to its high levels of fat (99.9%) and gross energy (7780 kcal/kg) soy lecithin may be used as a fat source for animals [Peña *et al.* 2014]. Pascual *et al.* [2000] reported that an increased inclusion of fat, and thus energy, in the diet for rabbit does improves milk yield and litter growth. Furthermore, the fatty acid composition of the fat source is very important as the content of polyunsaturated fatty acids (PUFA) in a diet can influence both ovarian and uterine function, while unsaturated fatty acids (UFA) elevate steroid hormones, such as estradiol, in preovulatory follicles, which may be beneficial for subsequent ovarian function [Zachut *et al.* 2008].

Heat stress is another crucial factor influencing reproductive and productive functions due to marked disturbances in animal biological functions as a result of feed intake depression [Marai *et al.* 2005], which would have deleterious effects on lactating does. The mean temperature in Egypt is about 34°C during the summer and 22°C during the winter. During summer the temperature may greatly exceed that of the comfort zone for rabbits (18-21°C), thus inducing a heat stress condition.

The objective of this study was to estimate the effect of SL supplementation for V-line rabbit does, during winter and summer seasons, on their productive and reproductive performance.

Material and methods

The present study was carried out at the Agriculture Research Center, Alexandria Governorate, from October to April (winter) and from May to September (summer). The experimental design was approved by the Animal Production Research Institute Scientific and Ethics Committee (Protocol number: 04-05-03-37).

Forty nulliparous, six month old, V-line doe rabbits were distributed in a completely randomized design and factorially arranged 4×2 with four dietary treatments and two seasons, with 10 experimental units. The same rabbit does were used in summer (mean body weight of 3583±240 g) and in winter (mean body weight of 3520±194 g). Rabbit does were mated three times with adult male rabbits that were not supplemented with SL. Rabbit does were housed in a naturally ventilated building and kept in individual

cages (60×55×40 cm) equipped with internal nest-boxes. Rabbits were given feed and water *ad libitum*. Rabbit does were fed the supplemented diets starting from one month before the day they were mated, until the 35th day of the 3rd lactation.

Treatments consisted of the control group (with no SL) and different SL inclusion levels in pelleted diets of 0.5, 1.0, and 1.5%, according to NRC [1977] recommendations (Tab. 1). The contents of dry matter (method 925.09), crude protein (method 999.04D) and ether extract (method 55-1976) of the diets were obtained as described by AOAC [2007]. The values of crude fibre, nitrogen free extract, digestible energy, calcium, available phosphorus, total methionine, total sulphur amino acid and total lysin of the diets were calculated according to the tables given by Sauvante *et al.* [2004], Gaafar *et al.* [2010], and Khaleel *et al.* [2014].

The determination of the SL fatty acid profile in the diets (Tab. 1) was performed according to Radwan [1978]. Air temperature (AT) and relative humidity (RH) were recorded daily using an electronic digital thermo-hygrometer.

The temperature humidity index (THI) was calculated using the equation modified by Marai *et al.* [2005]: $THI = AT - [(0.31 - (0.31 \times RH)) \times (AT - 14.4)]$. The THI values are classified as follows: absence of heat stress (THI < 27.8), moderate heat stress (THI = 27.8-28.9), severe heat stress (THI = 28.9-30.0) and very severe heat stress (THI ≥ 30.0).

The reproductive rhythm was semi-intensive according to Attia *et al.* [2009] and rabbit does were re-submitted to natural mating 11 d after delivery (43-d inter-pupping interval) using 20 bucks of 6 to 7 months of age. Bucks were kept under similar management and hygienic conditions and fed the same control diet of does. Mating was randomised, thus each female had the same chance to meet with the same male and vice-versa. Each rabbit doe was transferred to the buck's cage for mating (two services within 30 min by the same buck) and returned to its cage after copulation. Pregnancy was diagnosed by abdominal palpation at d 10 after mating and those shown to be non-pregnant were subjected to another mating until they became pregnant. The number of services per conception was calculated as the number of matings required to induce pregnancy at the 10th day after mating. Receptivity rate was calculated as the number of mated does divided by the number of mated does × 100. Conception rate was calculated as the number of pregnant does divided by the number of mated does × 100.

Body weight (BW) was recorded immediately after mating and after kindling, while daily feed intake was calculated from mating up to the end of the first week after parity, totaling five weeks, during three pregnancy periods. On the parturition day the doe was separated from the litter and she was taken to the nest only once a day in the morning for 15 – 20 minutes to nurse the kits. At this moment the doe was weighed before and after the nursing to determine the total milk production from the difference in body weight [Attia *et al.* 2011]. Milk production was measured from birth to the 35th day after parturition. Live litter size and kit weight at birth and at 35 days of age were also recorded. Economic efficiency was established based on the cost of the consumed ration (daily feed intake × price per kilogram of feed × 35 days).

Table 1. Ingredients and chemical composition of diets fed to V-line rabbits

Indices	Dietary soybean lecithin (g · kg ⁻¹)			
	0	5	10	15
Ingredients				
clover hay	400	400	400	400
yellow maize	100	87	70	55
barley	130	140	140	140
Wheat bran	150	163	180	200
soybean meal	175	170	170	165
soybean lecithin	0	5	10	15
molasses	30	20	15	10
dicalcium phosphate	8	8	8	8
sodium chloride	3	3	3	3
vitamin and mineral premix ¹	3	3	3	3
DL-methionine	1	1	1	1
Analysed and calculated composition (g · kg ⁻¹)				
dry matter	897	898	897	898
Crude protein	172	172	174	175
Crude fibre	143	144	145	147
ether extract	34	39	44	49
nitrogen free extract	560	553	544	545
digestible energy, MJ · kg ⁻¹	11.7	11.6	11.5	11.4
Ca	13.0	13.0	13.0	13.0
available P	3.6	3.6	3.6	3.6
total methionine	3.6	3.6	3.6	3.6
total sulphur amino acid	6.8	6.8	6.8	6.8
total lysine	9.3	9.3	9.3	9.3
Cost (US\$/kg)	0.14	0.14	0.14	0.14
Analysed fatty acid composition (g/kg of the total fatty acids)				
capric acid (C10:0)	63	36	71	46
lauric acid (C12:0)	61	51	3.6	4.7
myristic acid (C14:0)	48	10	47	57
palmitic acid (C16:0)	225	192	131	96
palmitoleic acid (C16:1n-7)	0.0	26	30	34
stearic acid (C18:0)	82	43	35	29
oleic acid (C18:1n-9)	300	291	264	247
linoleic acid (C18:2n-6)	180	283	389	458
arachidic acid (C20:0)	42	68	30	29
saturated fatty acid (SFA)	520	400	318	261
unsaturated fatty acid (UFA)	480	600	682	739
polyunsaturated fatty acid (PUFA)	180	283	389	458
monounsaturated fatty acid (MUFA)	300	317	294	281
SFA/UFA	1.1	0.7	4.7	3.5
MUFA/UFA	0.6	0.5	0.4	0.4
MUFA/PUFA	1.7	1.1	0.8	0.6
PUFA/UFA	0.4	0.5	0.6	0.6

¹Per kilogram contains: IU: vit A 6000, vit D₃ 450; mg: vit E 40, vit K₃ 1, vit B₁ 1, vit B₂ 3 mg vit B₃ 180, vit B₆ 39, vit B₁₂ 2.5, pantothenic acid 10; biotin 10, Folic acid 2.5, choline chloride 1200 Mg 15, Zn 35, Fe 38, Cu 5, Co 0.1, I 0.2 and Se 0.05.

Statistical analysis was performed using the SISVAR software [Ferreira 2011]. Data of rabbit does were analysed applying the linear model including the SL and season effects, and interaction effects between of them. Tukey's test was used to detect significant differences among the group means. The level of statistical significance was set at P<0.05.

Results and discussion

The average environmental temperature, relative humidity and THI were 22.3°C, 57.4% and 22.4 during the winter and 32.0°C, 31.3% and 29.9 during the summer, respectively. The obtained values in summer indicate heat stress, since the thermoneutral zone for rabbits is from 18 to 21°C [Habeeb *et al.* 1993].

There was no mortality among the rabbit does during the experimental period. There was no effect of the SL supplementation \times season interaction on the does' BW and feed intake ($P < 0.05$); however, SL supplementation at 1.5% increased the BW

Table 2. Effect of soy lecithin (SL) supplementation and season on body weight after mating and after kindling and feed intake of V-line rabbit does

Treatments	Rabbit does weight (g)		Feed intake (g/day)	Cost of feed (US\$)	Milk production (g/day)				
	after mating	after parturition			1 st week	2 nd week	3 rd week	4 th week	5 th week
	Effect of soy lecithin supplementation (%)								
0.0	3465 ^b	3312 ^c	220 ^a	1.08 ^a	106 ^c	158 ^c	163 ^b	101 ^b	55 ^b
0.5	3494 ^b	3407 ^b	212 ^b	1.04 ^b	115 ^b	180 ^{bc}	178 ^b	107 ^b	60 ^b
1.0	3504 ^b	3478 ^a	207 ^{bc}	1.01 ^{bc}	127 ^a	201 ^{ab}	195 ^a	119 ^a	70 ^a
1.5	2614 ^a	3516 ^a	202 ^c	0.99 ^c	131 ^a	204 ^a	206 ^a	123 ^a	73 ^a
Effect of the season									
Winter	3567	3477	206	1.01	122	197	192	115	68
Summer	3439	3344	217	1.06	116	160	170	106	55
Effect of the interaction of soy lecithin supplementation (%) by season									
0.0 × winter	3518	3326	216 ^b	1.06 ^b	104 ^d	163	173	103	58
0.5 × winter	3537	3457	208 ^c	1.02 ^c	115 ^b	190	181	109	62
1.0 × winter	3518	3530	204 ^c	1.00 ^c	131 ^a	215	201	125	75
1.5 × winter	3694	3598	195 ^d	0.95 ^d	136 ^a	219	214	124	78
0.0 × summer	3375	3288	227 ^a	1.11 ^a	110 ^d	149	142	95	48
0.5 × summer	3422	3323	218 ^{ab}	1.07 ^{ab}	114 ^{bc}	155	171	104	54
1.0 × summer	3480	3390	211 ^b	1.05 ^b	119 ^{bc}	166	179	107	57
1.5 × summer	3477	3377	213 ^b	1.03 ^b	121 ^b	169	189	119	61
SEM	12.79	17.13	1.55	0.01	0.82	1.63	1.24	0.77	0.71
P-values									
SL	0.040	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Season	0.004	0.001	0.001	0.001	0.010	0.001	0.001	0.011	0.001
SL × season	0.325	0.054	0.055	0.054	0.007	0.185	0.464	0.441	0.432

n=10 per treatment per parity per season.

ab... Means within a column not sharing common superscripts are significantly different.

of the rabbit does after mating (+4.3%) and after kindling (+6.1%) ($P < 0.05$). Diets supplemented with 1.0-1.5% reduced feed intake and cost of feeding ($P < 0.001$). In summer, BW was lower, while feed intake and feeding costs were higher ($P < 0.05$) (Tab. 2). SL supplementation provided enough energy to result in a lower feed intake of does and higher BW after mating and after kindling [Attia and Kamel 2012], since SL promotes the incorporation of fatty acids in micelles and increases fat absorption in the gut [Roy *et al.* 2010]. In summer, the difference in BW after mating and after parturition was 2.76%, compared to 2.52% in winter, probably because a part of the consumed energy is spent on heat dissipation [Marai *et al.* 2002].

The SL by season interaction influenced milk production in the first week, with a higher milk production in supplemented rabbits in both seasons (Tab. 2). In winter, SL increased milk production up to 30.77% and in summer, up to 10%. In summer, rabbit does may limit energy reserve mobilisation for milk production in an attempt to reduce the heat produced, thus the lower SL level (0.5%) was sufficient to improve milk production compared to the control group. In addition, secretion of thyroid hormones, T_3 and T_4 , is reduced during heat stress [Chiericato *et al.* 1995] and these are the hormones affecting milk synthesis.

The interaction of SL supplementation \times season was significant ($P < 0.05$); SL reduced the total SFA (-50%) and increased the total UFA (+97%) in milk of does provided with the 1.5% inclusion level during the winter and summer seasons (Tab. 3). The diet affects the fatty acid profile of rabbit milk. Milk of rabbit does consist of approximately 70.4% total SFA, 12.8% total MUFA and 15.6% total PUFA [Maertens *et al.* 2006]. These values are close to the ones recorded in this study. The composition of milk in does receiving diets with 1.5%, in both seasons, was 64.16% SFA, 19.97% MUFA and 15.87% PUFA (-50% SFA, +54% UFA, and +154% PUFA, compared to the milk composition in the control group). SL supplementation decreased SFA and increased UFA in the milk due to the intake of rations with SL, resulting in milk by 53.91% richer in UFA compared to the control diet.

In summer (heat stress), the total SFA level increased and total levels of UFA, PUFA and MUFA were reduced in milk compared to the winter. A high ambient temperature may result in a decreased activity of the enzymes acetyl-CoA-carboxylase and stearoyl-CoA-desaturase in the adipose and liver tissues and consequently, cause a reduction in the synthesis of fatty acids, specifically, the synthesis of MUFA from SFA, respectively [Kouba *et al.* 1999].

From the second to the fifth week of the lactation period milk production was higher with SL inclusion at 1.0-1.5% and during the winter season (Tab. 4). Maertens *et al.* [2006] reported that the number of suckling kits may increase milk production, leading to heavier kits during the lactation period. If doe rabbits have a good body condition and can better utilise the ingested energy, they will have a higher energy supply for milk production; consequently, this results in higher kit weight at weaning.

The negative effect of the summer season on productivity of does may be due to the low metabolisable energy reserves left for growth, as a consequence of greater

Table 3. Effect of soy lecithin (SL) supplementation and season on fatty acids profiles in milk of V-line rabbit does

Treatments	Fatty acid profile of the milk (%)									
	C12:0	C14:0	C16:0	C18:0	C18:2	C18:3	C20:0	SFA	UFA	MUFA
	Effect of soy lecithin supplementation (%)									
0.0	3.93 ^a	2.86 ^b	16.65 ^a	16.13 ^a	10.51 ^d	0.94 ^d	0.47 ^b	75.57 ^a	24.43 ^d	11.45 ^d
0.5	3.65 ^b	2.98 ^b	14.77 ^b	14.10 ^b	12.00 ^c	1.48 ^b	0.51 ^b	70.84 ^b	29.16 ^c	13.48 ^e
1.0	3.91 ^a	3.41 ^a	13.84 ^c	13.04 ^d	12.77 ^b	1.15 ^c	0.49 ^b	67.79 ^c	32.21 ^b	18.27 ^b
1.5	3.35 ^c	3.09 ^b	12.44 ^d	13.67 ^c	13.89 ^a	1.97 ^a	0.71 ^a	64.16 ^d	35.54 ^a	15.87 ^a
Winter	3.29	4.35	14.02	12.65	11.92	2.02	0.75	68.91	31.09	13.94
Summer	4.12	1.83	14.82	15.82	12.67	0.75	0.34	70.27	29.73	13.42
Effect of the interaction of soy lecithin supplementation (%) by season										
0.0 × Winter	3.79 ^a	4.48 ^{ab}	15.78 ^b	14.05 ^d	10.80 ^e	1.34 ^c	0.64 ^b	74.64 ^b	25.35 ^d	12.14 ^c
0.5 × Winter	2.74 ^e	3.77 ^c	14.35 ^c	12.06 ^e	11.44 ^d	2.25 ^b	0.66 ^b	70.55 ^c	29.44 ^c	13.67 ^b
1.0 × Winter	3.18 ^d	4.81 ^a	13.51 ^d	12.06 ^e	12.20 ^c	1.47 ^c	0.65 ^b	66.92 ^d	33.08 ^b	13.69 ^b
1.5 × Winter	3.47 ^c	4.31 ^b	12.46 ^e	12.41 ^e	13.23 ^b	3.02 ^a	1.05 ^a	63.53 ^c	36.47 ^a	20.22 ^a
0.0 × Summer	4.07 ^b	1.24 ^e	17.51 ^a	18.19 ^a	10.22 ^d	0.53 ^d	0.31 ^c	76.49 ^a	23.51 ^e	10.75 ^e
0.5 × Summer	4.55 ^a	2.18 ^d	15.18 ^b	16.14 ^b	12.55 ^c	0.71 ^c	0.35 ^c	71.13 ^b	28.87 ^d	14.17 ^c
1.0 × Summer	4.65 ^a	2.01 ^d	14.17 ^c	14.01 ^d	13.34 ^b	0.81 ^{cb}	0.34 ^c	68.67 ^c	31.32 ^c	13.27 ^d
1.5 × Summer	3.23 ^c	1.88 ^d	12.42 ^{de}	14.93 ^c	14.57 ^a	0.93 ^b	0.36 ^c	64.78 ^d	35.22 ^b	15.49 ^b
SEM	0.09	0.12	0.18	0.18	0.13	0.04	0.03	0.17	0.17	0.13
P-values										
SL	0.001	0.002	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Season	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
SL × season	0.001	0.001	0.002	0.001	0.001	0.001	0.001	0.006	0.006	0.001

*n = 3 per treatment per season.

ab... Means within a column not sharing common superscripts are significantly different.

C12:0 – lauric acid; C14:0 – myristic acid; C16:0 – palmitic acid; C18:0 – stearic acid; C18:2 – linoleic acid; C18:3 – linolenic acid; C20:0 arachidic acid; SFA – saturated fatty acids; UFA – unsaturated fatty acids; MUFA – monounsaturated fatty acids.

energy expenditure due to an increased respiration rate at hot ambient temperature [Habeeb *et al.* 1993].

Receptivity and conception rates increased, while the number of services per conception decreased by SL supplementation as compared with the control diet. In summer, service number increased and conception rate was reduced. Kit weight at birth and at weaning was higher ($P < 0.05$) when the does received dietary SL at 1.0-1.5% and when they were reared in the winter. The interaction of SL supplementation

Table 4. Reproduction traits of rabbit does during three mating periods and performance of their offspring during three lactation periods

Treatments	Rabbit does			Offspring		
	receptivity rate (%)	conception rate (%)	number of services	at birth	at weaning	
				size	weight (g)	size
				weight (g)	supplementation (%)	
0.0	75.3 ^b	64.4 ^b	1.54 ^a	7.14 ^e	49.8 ^e	5.59 ^e
0.5	82.2 ^{ab}	71.3 ^{ab}	1.28 ^b	7.75 ^b	54.0 ^b	6.31 ^b
1.0	88.1 ^a	79.2 ^a	1.21 ^b	8.94 ^a	57.2 ^a	7.71 ^a
1.5	90.1 ^a	81.2 ^a	1.19 ^b	8.92 ^a	57.5 ^a	7.64 ^a
				Effect of the season		
Winter	85.6	76.4	1.28	8.80	56.3	7.31
Summer	80.0	68.3	1.37	6.73	50.7	5.65
				Effect of the interaction of soy lecithin supplementation (%) by season		
0.0 × winter	77.5	66.2	1.52	7.52 ^e	52.3	5.93 ^e
0.5 × winter	84.5	73.2	1.27	8.28 ^b	56.1	6.70 ^b
1.0 × winter	88.7	81.7	1.18	9.59 ^a	58.1	8.25 ^a
1.5 × winter	91.6	84.5	1.15	9.82 ^a	58.5	8.35 ^a
0.0 × summer	70.0	60.0	1.60	6.23 ^d	43.7	4.80 ^d
0.5 × summer	76.7	66.7	1.34	6.50 ^d	49.1	5.40 ^e
1.0 × summer	86.7	73.3	1.27	7.40 ^e	54.8	6.44 ^b
1.5 × summer	86.7	73.3	1.27	6.80 ^{cd}	55.1	5.97 ^{bc}
SEM	1.83	2.18	0.03	0.09	0.31	0.08
				P-values		
SL	0.005	0.008	0.001	0.001	0.001	0.001
Season	0.075	0.037	0.028	0.001	0.001	0.001
SL × season	0.888	0.958	0.972	0.001	0.085	0.001

n=10 per treatment per parturition per season.

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× season influenced ($P<0.05$) the size of the offspring at birth and at weaning; 1.0-1.5% SL inclusion resulted in a greater offspring size in both seasons (Tab. 4).

Supplementation with PUFAs in diets for cows increased the total follicular number and the size of the dominant or pre-ovulatory follicle [Bilby *et al.* 2006]. Rebollar *et al.* [2014] reported an increase in luteal hormone secretion after ovulation induction in PUFA n-3 supplemented does, which could imply greater ovarian follicular development and steroid production. Elkomy and El-Spei [2015] reported that rabbit does supplemented with a source of PUFA exhibited a higher concentration of 17- β estradiol and the same $\text{PGF}_{2\alpha}$ concentration as compared to rabbits that received eCG + $\text{PGF}_{2\alpha}$, before artificial insemination. Due to the higher estradiol concentration, receptivity rate was also higher, similarly as litter size and bunny BW at birth.

SL supplementation resulted in greater kit weight at birth and at weaning, reflecting the better body condition of the rabbit does. During the winter season, kit weight was higher at birth and at weaning, comparing with the summer when oocyte growth and embryo development might fail due to the high environmental temperature and result in a higher embryo mortality [Hansen 2007]. The interaction between SL inclusion and season was significant ($P<0.05$) for litter size at birth and at weaning. Litters from rabbit does fed diets with 1% to 1.5% SL were heavier????more numerous?????. The PUFA consumption may increase the secretion of the luteal hormone and 17- β estradiol, thus increasing the litter size [Rebollar *et al.*, 2014; Elkomy and El-Speiy, 2015]. In addition, SL may improve fat absorption and energy use by does and their kits. During pregnancy and in the first days of lactation both the developing fetuses and offspring are dependent on the mother for nutritional requirements, growth, development and pregnancy outcome [Rebollar *et al.* 2014].

In conclusion, the addition of 1.0-1.5% soybean lecithin improved the productive and reproductive traits for V-line rabbit does and growth performance of their kits up to weaning in the winter and summer seasons.

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