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Effect of dietary fat sources on productive performance, milk yield and milk composition of multiparous rabbit does

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ABSTRACT

The present study was conducted to evaluate the effect of adding different sources of fat or oil on productive performance, milk yield and milk composition of rabbit does. Thirty five multiparous New Zealand White rabbit does (two years old) with initial body weight of 3.5 – 4.0 kg were obtained from a local commercial source. Rabbit does were distributed into 7 groups (5 does in each), a control and 6 experimental groups according to the source of fat or oil used. Rabbit does were naturally inseminated using NZW buck rabbits (one buck/ 3 does) fed the control diet of lactation. Experimental period extended for about 60 days from the beginning of gestation period of does to weaning period of litters. Productive parameter of rabbit does including numbers of litters, average live body weight, weight gain, litter mortality rate and milk yield were recorded. At 13th day of lactation period, milk samples were collected manually from all does of each group and analyzed for basic chemical composition and essential fatty acids profile (linolenic and linoleic acids). Results revealed that the highest total number of litters born alive at birth were recorded in group B and G (47 & 45) fed on diets supplemented with fish oil and polyfat respectively, while the lowest total number of litters born alive at birth were recorded in group F (35) in which rabbit does fed diet supplemented with palm oil plus lysoforte. The highest total number of litters at 21 and 30 days of age was recorded in the group fed diet supplemented with 3% fish oil (40) followed by group G (37). The lowest mortality rate were reported in groups fed diets supplemented with sunflower oil, palm oil and fish oil (7.5, 12.82 and 14.89%) respectively, in comparison with the control group (25.0%). At 21 and 30 days of lactation, polyfat supplemented group had the greatest litter weight (233.4±11.7g & 404.1g), while sunflower oil supplemented group recorded the lowest weight of litters (209.7±4.8g & 365.6g). Milk yield of the groups fed diets containing 3% sunflower oil, animal fat, palm oil and fish oil were increased by about 17.7, 16.4, 15.9 and 15.8 % respectively than that recorded in control group. Addition of different fat sources especially sunflower oil to the diets of rabbit does significantly increased milk yield, fat and essential fatty acid contents of milk as well as beneficially improved milk composition traits.

Key words: Fat sources, productive performance, milk yield, composition, rabbit doe

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1. INTRODUCTION:

Rabbits are now one of the most popular mammalian domestic pet; they are an extremely important livestock species and laboratory model worldwide. In the recent years more attention has been given in selection strategies to obtaining robust animals that have good reproduction and survival characteristics. Lipids (fats or oils) as energy carriers and sources of essential unsaturated fatty acids have attracted the attention of nutritionists in recent years. Fats are very energy dense raw materials. Enriching complete diets with components high in PUFA makes it possible to program the fatty acid profile of milk as a result of transferring certain components from the feed. Many experiments attempted to increase milk yield by using high-energy rations with increased carbohydrate content but failed to produce anticipated results.

Among the many nutrients used in feeding, special attention has recently been given to the quantity and quality of fats (Kowalska, 2008). Because rabbit diets normally have a fibrous nature, fats show potential for increasing energy concentration (Maertens, 1998). Milk yield and milk components determine the rate of growth of the newborn rabbits (Khalil et al., 2004). There are many traits correlated with rabbit milk production and its composition such as litter size at weaning, litter weight at weaning or mortality (Mehaia et al., 2002 and Al-Sobayil et al., 2005). Rabbit does are in general allowed to nurse their kits till weaning age (4-5 weeks of age). Kits are until 18-19 days of age exclusively depending on the milk of their mother (Fortun-Lamothe & Gidenne, 2000).

The ability of females to produce milk is one of the main factors involved in after-birth growth rate of young and in the determination of litter size at weaning (Baselga et al., 1982). Rabbit milk fat contains triacylglycerols of medium chain fatty acids (MCFA) that are synthesized in the mammary gland and represent approximately one half of triacylglycerols in milk (Jones & Parker, 1981). Consequently, information relating with milk yield and composition remains relatively scarce. Few studies have been carried out concerning milk production and its composition in rabbits respects other species, because milk sample collection and recording milk production are difficult. The present study was carried out to investigate the effect of adding different sources of fat or oils to rabbit does diets on productive performance including numbers of litters, average live body weight, weight gain, litter mortality rate as well as milk yield and milk composition.

2. MATERIALS AND METHODS:

2.1.Experimental animals and housing:

Thirty five multiparous New Zealand White rabbit does (two years old) with initial body weight of 3.5 - 4 kg were obtained from a local commercial source. Does were distributed randomly into 7 groups (5 does in each) and assigned into seven different dietary treatments. Rabbit does were housed separately in individual galvanized wired cages (50 x 60 x 35 cm) and arranged in

double tier batteries allocated in two rows till insemination. After insemination, rabbits does were placed again in the same individual cages and arranged in double tier batteries allocated in two rows. Nest boxes (30 x 25 x 30 cm) were attached to the front sides of the cages five days prior to kindling and removed at 30 day of lactation (weaning age). The bucks were housed in individual cages as that of females, but without nest boxes. All cages were equipped with feeders (made of galvanized steel sheets) and nipples (automatic drinkers). Rabbit does were naturally inseminated using NZW buck rabbits (one buck/3 does) fed on the control diets of lactation. Pregnancy diagnosis was done by palpation at 10-12 days post-mating and does fail to conceive posts 1st mating were re-inseminated. Rabbits in all treatment groups were kept under similar managerial system and environmental conditions. The biosecurity and hygienic conditions inside the farm were very important before the beginning of the experiment.

2.2.Experimental design:

The following feeding program was applied in this experiment for seven experimental groups. Experimental period extended for about 60 days from the beginning of gestation period of does to weaning period of kits. The experimental design and distribution of rabbit does among the different experimental groups were shown in Table (1).

Table (1): Suggested experimental design of the current work

Treatments	Control	Fish oil (FO)	Sunflower oil (SFO)	Animal fat (AF)	Palm oil (PO)	Palm oil + Lysoforte*	Polyfat **
Groups	A	B	C	D	E	F	G
Number of does	5	5	5	5	5	5	5
Level of animal fat & oil (%)	-	3	3	3	3	3	3

*A natural absorption enhancer and natural bio surfactant enriched in lyso-phosphophatidylcholine (LPC).

** Polyfat composed of: palm oil FFA (75%) + soya oil (5%) + soft oil FFA (20%).

2.3. Diets and Feeding:

Two standard control pelleted control diets, one for pregnancy and other for lactation (D_1 & D_2) were formulated from yellow corn, soybean meal, wheat bran, berseem hay and molasses along with other ingredients to meet minimum nutrient requirements of rabbit does according to NRC (1977). Two experimental pelleted diets for pregnancy and lactation (D_3 & D_4) were formulated from the same feed ingredients and contain different sources of fat or oil (fish oil, sunflower oil, animal fat and

palm oil, palm oil+ lysoforte and polyfat) at the level of 3%. Physical and calculated chemical composition of the pelleted control and experimental diets are presented in Table 2.

Rabbit does were distributed into 7 groups, a control and 6 experimental groups according to the source of fat or oil used. Does in the first group were fed on basal control pelleted diets (D_1 & D_2) without fat or oil supplementation during pregnancy and lactation periods. This group was assigned as a control to which the other treated groups were compared. Animals in the second, third, fourth and fifth groups were fed on the pelleted experimental diets (D_3 & D_4) containing 3% fish oil, sunflower oil, animal fat and palm oil. Rabbit does in the sixth and seventh groups fed on the same experimental diets (D_3 & D_4) supplemented with 3% palm oil+ lysoforte and polyfat.

Table (2): Physical and chemical composition of control and experimental diets.

Ingredients	Diets			
	Control diets		Experimental diets	
	Pregnancy (D_1)	Lactation (D_2)	Pregnancy (D_3)	Lactation (D_4)
Physical composition				
Yellow corn	29.0	26.0	26.0	17.0
Soybean meal	10.6	17.0	12.6	16.8
Wheat bran	13.0	11.0	5.0	10.0
Berseem hay	43.8	42.4	49.8	49.6
Molasses	2.0	2.0	2.0	2.0
Oil	-	-	3.0	3.0
Common salt	0.50	0.50	0.50	0.50
Di-calcium phosphate	0.55	0.55	0.55	0.55
Methionine	0.25	0.25	0.25	0.25
Premix*	0.30	0.30	0.30	0.30
total	100	100	100	100
Chemical composition				
Dry matter	90.13	88.64	87.53	87.62
Crude protein	16.03	18.08	16.28	18.05
DE(kcal/kg)	2601	2609	2666	2640
DE(MJ/kg)	10.88	10.91	11.15	11.05
Crude fiber	13.44	13.16	14.08	14.59
Ether extract	2.94	2.87	5.59	5.56
NFE	48.49	46.56	44.62	42.19
Calcium	0.78	0.79	0.87	0.88
Total phosphorous	0.49	0.50	0.43	0.48
Methionine	0.60	0.67	0.67	0.69

* (Multivita company), Each 3 kg premix contains: 1 kg vitamins (Vit.A 8000000 IU; Vit. D3, 400000 IU; Vit. E 30000mg; calcium carbonate material carrier up to 1kg) and 2kg minerals (Zinc 50000 mg; Manganese 50000 mg; Iron 50000mg; Copper 10000; Iodine 500 mg; Selenium 200mg; Cobalt 100mg; calcium carbonate material carrier up to 2kg).

2.4. Measured parameters:

2.4.1. Productive performance:-

2.4.1.1 Numbers of litters:

Numbers of litters from each rabbit doe in all groups were individually recorded at the day of birth and at 21 and 30 days of age, and then the total litter number for each group was calculated.

2.4.1.2. Average live body weight of litters:

Live body weight of litters from each rabbit doe was individually recorded at the day of birth and at 21 and 30 days of age. Individual live body weight was totaled as divided by the number of litters to obtain the average LBW for each group.

2.4.1.3. Litter mortality rate:

Litter dead for each rabbit doe in all experimental groups and control was recorded during the suckling period, then mortality rate was calculated during the whole experimental period.

2.4.1.4. The milk yield:

The daily milk yield of each rabbit doe was measured by determining the weight difference of the doe before and after nursing. This weight-suckle-weight method is widespread used for research purposes and has an advantage over weighing of the kits. Kit weighing is more difficult because they are nervous and the accuracy is lower because kits show some urine losses even during suckling event (Lebas, 1971).

2.4.1.5. Milk composition:-

At the peak of milk production (13^{th} day of lactation period), milk samples were collected manually from all does of each group by gently hand milking of all active teats after injection the females with 3-5 IU oxytocine in order to stimulate the milk ejection. Milk samples were stored at –20 ˚C till further analysis. Basic chemical composition of milk samples including crude protein, fat, total solid, solid non-fat, ash and pH was determined by using Milko-scan (Model 133B). Milk composition was undertaken according to AOAC (1994). Higher fatty acids profile (linolenic and linoleic acid) of milk was analyzed by using gas chromatography (Anonymous, 1996).

2.4.1.6.Statistical analysis:

Experimental raw data subjected to statistical analyses, from which means and standard errors were calculated. Differences were tested for significance by the one-way analysis of variance using procedure of Statistical Analysis System (SPSS, 2008). The significant differences ($P<0.05$) among treatment means were tested using Duncan's multiple range test (1955). Values of $P < 0.05$ were considered significant differences.

3.RESULTS & 4. DISCUSSION:

The highest total number of litters born alive at birth were recorded in group B and G (47 & 45) fed on diets supplemented with fish oil and polyfat respectively, while the lowest total number of litters born alive at birth were recorded in group F (35) in which rabbit does fed diet supplemented with palm oil plus lysoforte. The total number of litters born alive at birth in group C fed on diet supplemented with sunflower oil was similar to control one (40) as shown in Table 3 and figure 1. At 21 and 30 days of lactation period, the total number of litters was constant. The highest total number of litters was recorded in the group fed diet supplemented with 3% fish oil (40) followed by group G (37). The lowest value was recorded in group fed on palm oil plus lysoforte (28). The total number of litter of all treated groups were higher than that recorded by control except the groups D and F fed on diets supplemented with 3% animal fat or 3% palm oil plus lysoforte. Similar results were reported by Maertens & De Groote (1988) who observed a higher number of

litters born alive per does given high-energy diets. Moreover, Pascual et al. (1999) found that feeding high fat diets for multiparous rabbit does resulting in an increased number of litters born alive and decreased the number of litters replaced during lactation period. The positive effect of dietary fat on the number of litters born alive may be attributed to fats considered as the main source of polyunsaturated fatty acids. These PUFA present in fish products (fish oil) and when included in the rabbit female diets could be a way to improve the reproduction and productivity of these animals as PUFA are involved in both prostaglandin (PG) and steroid metabolism (Wathes et al., 2007).

The results revealed that mortality rate was numerically lower for all experimental groups in comparison with control during suckling period as illustrated in Table 4 and figure 2. The lowest mortality rate were reported in groups C, E and B fed on diet supplemented with sunflower oil, palm oil and fish oil (7.50, 12.82 and 14.89%) respectively, in comparison with control (25.00%). These results are in agreement with that reported by Fernandez-Carmona et al. (1996) who found that, high fat diets for rabbit breeding does housed at 30°C increased the young survival rate. Also, Kowalska (2008) found that supplementation of female rabbit diets with 3% fish oil decreased the mortality rate of litters. On the contrary, Lebas & Fortun-Lamothe (1996) demonstrated that high-fat content in the diet of rabbit does resulting in negative effect on mortality of litters. The positive effect of dietary fat on young survival rate may be attributed to an increased level of essential fatty acids in the rabbit milk as result of addition of fats to does diets (Christ et al., 1996). Fortun-Lamothe & Boullier (2004) found that the amount and type of dietary fat, especially the level of n-3 and n-6 PUFAs, can modulate immune function both at systemic and intestinal levels, as fatty acids are structural components of cell membranes and signaling molecules and precursors for the synthesis of eicosanoids and either excess or deficiency of them could be harmful to the immune system.

Results in Table 5 revealed that, there were no significant differences in the litter initial weight (at birth) between all experimental groups and control one. At the end of the 3rd week of lactation, polyfat supplemented group had the greatest body weight (233.4±8.74g) while, sunflower oil supplemented group had the lowest value (209.7±4.8g). At the end of the suckling period (30 days), litters in group G recorded the highest body weight and best cumulative body weight gain (404.1±7.57 & 346.8±6.21g) followed by litters in group E (398.8±8.47 & 344.0±7.56g) in comparison with the control (331.3±9.87 & 275.8±10.75g g). Similar results were recorded by Maertens & De-groote (1988), Fernandez-Carmona et al. (1996), Cervera & Fernandez-Carmona (1997) and Pascual et al. (1998 & 1999) who found that high fat diets improved significantly the productive parameters of rabbit does including litter weights and litter gain at weaning. Kowalska (2008) reported that female rabbits fed a complete diet with 3% fish oil had higher body weight of litters at birth, 21 and 35 days of age. Rebollara et al. (2014) reported that dietary supplementation of rabbit does with PUFA n-3 produced greater litter weight at parturition and at weaning. On the other hand, Fortun-Lamothe & Lebas (1995), Odunsi (1999), Maertens et al. (2005) and Eiben et al. (2010) found that, no differences in litter performance during

lactation related to dietary fat supplementation. The enhancement effect of dietary fat on body weight development and weight gain of litters may be attributed to stimulate the energy intake and consequently the milk yield. This fact obviously induces a higher litter weight gain and a heavier weight at weaning with positive effects on the health and performance of the growing rabbits. High energy diets can increase the milk production and therefore the weight of litters at weaning in comparison with the more commonly available less concentrated diets (Xiccato et al., 1995; Pascual et al., 2000 and Parigi-Bini et al., 1996).

The effect of dietary fat supplementation on average milk yield of rabbit does at 13 days of lactation period and daily milk intake of litters per day is presented in Table 6 and figure 4. The statistical analysis of data revealed that the addition of different sources of fat to rabbit does diet increased significantly ($p<0.007$) the average milk yield in all experimental groups as compared with the control one. There were no significant differences in daily milk intake of litters per day among all experimental groups supplemented with different sources of fat. Milk yield of the experimental groups fed diets containing 3 % sunflower oil, animal fat, palm oil and fish oil were increased by about 17.7, 16.4, 15.9 and 15.8 % respectively than that recorded by control. These results agreed with the findings reported by Parigi-Bini et al. (1996) who found that rabbit does fed diet containing 3 % animal fat had a higher milk production ($p<0.05$). Feeding of fat-added diet to semi-intensively reared does will improve their energy deficit and thus the body mobilization because of a greater milk production. High energy diets seem to accentuate body reserve mobilization, as they stimulate primarily milk production of the rabbit does. Fat additions to doe diets allow an increase of the DE intake and milk production but it failed to reduce the negative energy balance of the primiparous doe simultaneously pregnant and lactating (Fortun-Lamothe, 1997 and Fortun-Lamothe et al., 1999). On the other hand, Kowalska (2008) found that addition of 3% fish oil to the female rabbit diets had no significant effect on the milk production.

Table 7 revealed that addition of fat to the diet of rabbit does significantly ($p<0.01$) increased fat content of milk compared with control. The highest fat % of milk was recorded in does fed on 3% palm oil supplemented diet (18.56%) followed by group D received diet had 3% animal fat (18.40%), while the lowest value was recorded in control group (12.76%).Crude protein, SNF and total solid were significantly increased by dietary fat supplementation in all treated groups. Rabbit does fed on 3 % sunflower oil supplemented diet recorded highest total solid (39.16%) compared with control (29.67%), while group fed on diet supplemented with 3 % palm oil recorded highest milk protein (13.50%). No significant differences were observed in lactose % and pH of doe's milk between all experimental groups and control. Similar results were recorded by Christ et al. (1996) and Pascual et al. (1996) who found that the inclusion of fat in rabbit does diets at high concentration increased the milk fat content at 7 and 28 days of lactation. Our results also are in accordance with those recorded by Parigi-Bini et al. (1996) and Pascual et al. (1999) who found that fat addition modify the rabbit milk composition especially fatty acids proportions.

linolenic acid (C18:3) content of rabbit does milk significantly increased ($p<0.001$) in all experimental groups when compared with control as shown in Table 7. The highest value of linolenic acid was recorded in group C fed diet supplemented with 3 % sunflower oil (0.47 mg/ml) followed by group G (0.43 mg/ml), while the lowest value was reported in group E (0.18 mg/ml) compared with control (0.089 mg/ml). Regarding to linoleic acid (C18:2), does milk in groups C and B fed on diet supplemented with 3 % sunflower oil and 3 % fish oil had significantly ($p<0.01$) higher linoleic acid (0.45 & 0.34 mg/ml respectively) than that recorded by control (0.11 mg/ml). Our results are in accordance with those reported by Lebas et al. (1996) and Christ et al. (1996) who found that dietary sunflower oil or rapeseed oil increased the unsaturated fatty acid content of the rabbit milk. Generally, unsaturated fatty acid content of the milk increase when vegetable oil was added to the diet. Additionally, medium chain fatty acids content (C8 to C15) decrease, while long chain fatty acids content (C16 to C22) increases in the milk of does given a diet containing fat.

Table 3. Total number of litters during different lactating periods

Groups		At birth	At 21day	At 30day
A	Control	40	30	30
B	Fish oil	47	40	40
C	Sunflower Oil	40	36	36
D	Animal fat	38	29	29
E	Palm Oil	39	34	34
F	Palm oil + Lysoforte*	35	28	28
G	Polyfat**	45	37	37

* A natural absorption enhancer and natural biosurfactant enriched in lyso phosphophatidyl choline (LPC).
** Polyfat composed of: palm oil FFA (75%) + soya oil (5%) + soft oil FFA (20%)

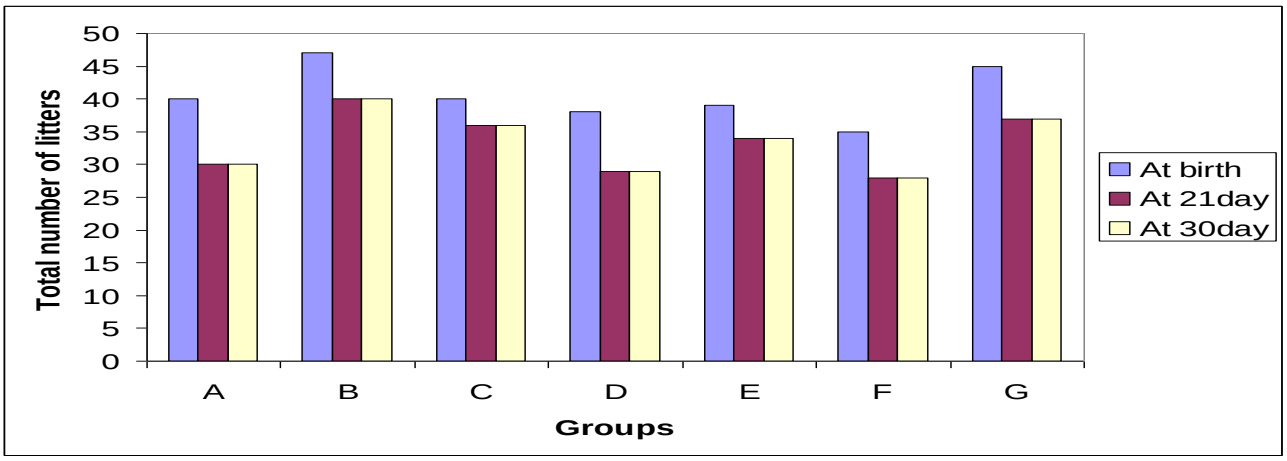

Figure (1): Total number of litters during different lactating periods

Table 4. Mortality rate of litters during the lactating period

Period (week)	Groups						
	A	B	C	D	E	F	G
0-1	8	6	2	4	2	5	6
1-2	-	-	1	2	2	3	2
2-3	2	1	-	3	1	-	-
3-4	-	-	-	-	-	-	-
Total (0-4)	10	7	3	9	5	8	8
Mortality rate (%)	25.00	14.89	7.50	23.68	12.82	22.86	17.78

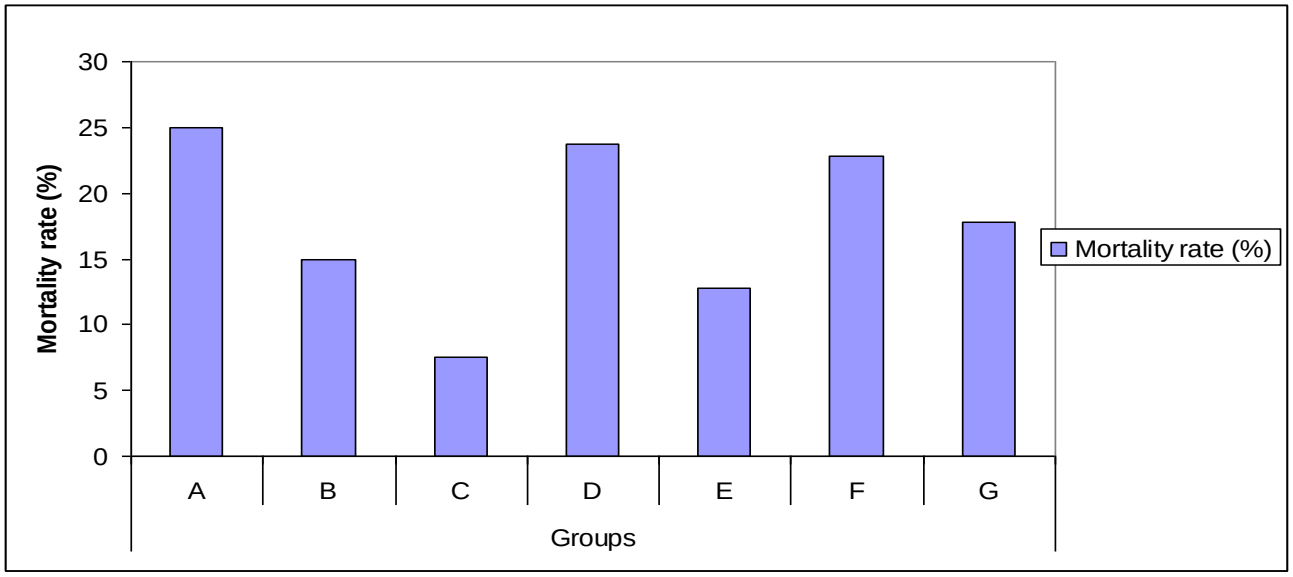

Figure (2): Mortality rate of litters during the lactation period

Table 5. Effect of different dietary fat sources on the live body weight and weight gain (g) of litters (means±SE)

Item	A	B	C	D	E	F	G	P
Initial litter weight (at birth)	55.5± 2.64	59.0± 2.13	55.5± 2.02	52.0±2. 36	54.8±1.8 0	54.7±1. 78	57.3±2.7 5	0.46
Litter weight at day 21	186.8 ± 11.68^{c} *	217.2 ± 3.40^{ab}	209.7 ± 4.8^{b}	220.4± 5.9 ^{ab}	215.0± 6.45 ^{ab}	231.9± 6.27 ^{ab}	233.4± 8.47^{a}	<0.01
Weight gain 0-21	131.3 ± 12.26^{c}	158.2 ± 4.48^{ab}	154.2 ± 5.19^{b}	168.4± 5. 99 ^{ab}	160.2± 5.67 ^{ab}	177.2± 6.56^{a}	176.1± 7.22^{a}	<0.01
Litter weight at day 30	331.3 ± 9.87^{c}	395.7 ± 7.14^{a}	365.6 ± 12.5^{b}	390.8± 4.5^{a}	398.8± 8.47^{a}	392.6± 6.52^{a}	404.1± 7.57^{a}	<0.01
Weight gain from 21-30 day	144.5 ± 17.44^{b}	178.5 ± 8.64^{a}	155.9 ± 11.84^{a} ^{b}	170.5± 6.43^{ab}	183.8± 8.18^{a}	160.6± 6.46^{ab}	170.7± 8.5^{ab}	<0.01
Weight gain from 0-30 day	275.8 ± 10.75^{c}	336.7 ± 7.77^{a}	310.1 ± 13.39^{b}	338.8± 5.03^{a}	344.0± 7.56^{a}	337.9± 7.12^{a}	346.8± 6.21^{a}	<0.01

*Means with different superscripts in the same raw are significantly different (p<0.05).

Table 6. Milk yield of rabbit does at 13th day of lactation period (means ± SE)

Item	A	B	C	D	E	F	G	P
Mean number of litters	6.40±051	8.20±0. 73	7.60± 0.81	6.40± 0.81	7.00± 0.89	6.60± 0.40	7.40± 0.87	
Average milk yield g/day (13th day)	176.0± 5.09 ^{b*}	209.0± 7.8^{a}	213.8± 10.07 ^{a}	210.6± 7.35 ^{a}	209.4± 1.69 ^{a}	191.2 ± 7.55 ^{ab}	200.0 ± 6.52^{a}	0.007
Daily milk intake g/kit/day	27.5±2.2 5	25.5±1. 87	28.1± 3.78	32.9± 5.44	29.9± 5.14	28.9± 2.38	27.0± 3.65	0.65

*Means with different superscripts in the same raw are significantly different (p<0.05).

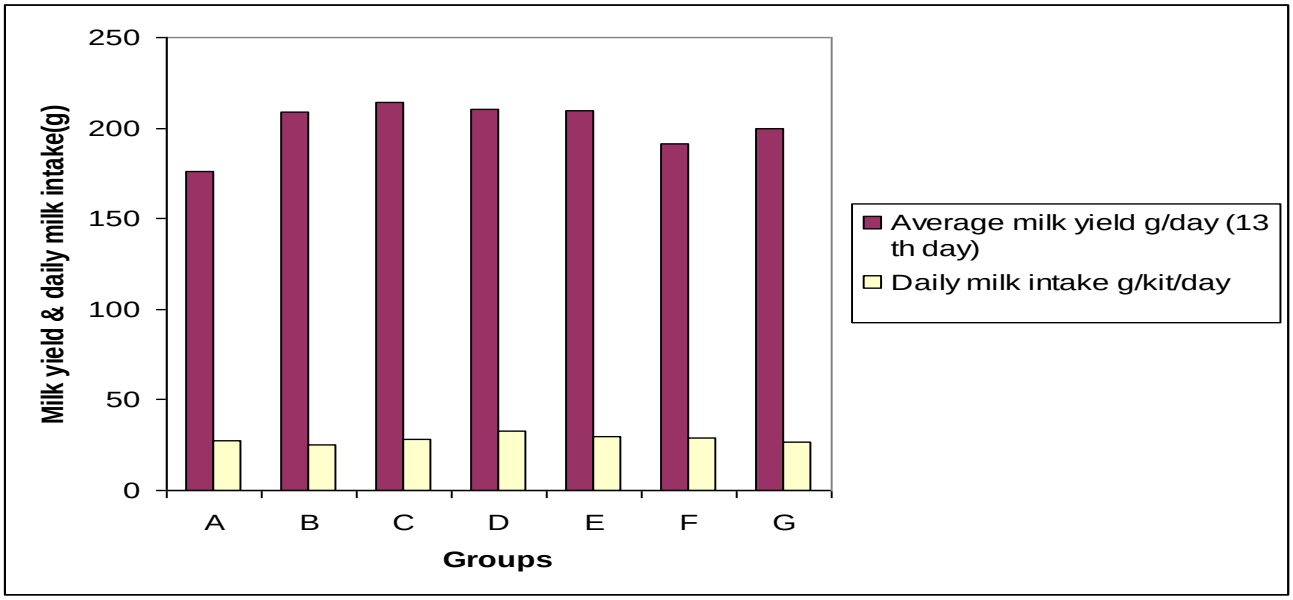

Figure (3): Milk yield of rabbit does at 13th day of lactation period

Table 7. Milk composition of lactating rabbit does as influenced by dietary treatments

Item	A	B	C	D	E	F	G	P
Fat	12.76± 0.37^{c}*	18.36± 0.18^{a}	18.37± 0.28^{a}	18.4± 0.49^{a}	18.56± 0.14^{a}	16.53± 0.2^{b}	18.13± 0.37^{a}	<0.01
Protein	12.50± 0.06^{d}	12.63± 0.09^{cd}	13.03± 0.09^{abc}	13.30± 0.2^{ab}	13.50± 0.15^{a}	12.86± 0.23^{bcd}	13.00± 0.15^{bc}	0.005
Lactose	1.60± 0.05	1.80±0. 06	1.81± 0.07	1.88± 0.15	1.74± 0.15	1.62±0. 08	1.77±0. 09	0.48
SNF	16.14± 0.2^{d}	16.2±0. 11^{cd}	16.61± 0.21^{bcd}	17.64± 0.13^{a}	17.17± 0.14^{ab}	16.79± 0.29^{b}	16.77± 0.08^{bc}	0.001
Total solid	29.67± 0.5^{d}	35.63± 1.45^{b}	39.16± 0.26^{a}	35.94± 0.39^{b}	35.67± 0.16^{b}	32.36± 0.19^{c}	36.23± 0.5^{b}	<0.01
pH	5.52± 0.07 ^{abc}	5.34± 0.07 ^{bc}	5.60± 0.07 ^{ab}	5.70± 0.06 ^{a}	5.38± 0.14 ^{abc}	5.24±0. 07^{c}	5.41±0. 18 ^{abc}	0.08
Linolenic acid (mg/ml)	0.089± 0.01^{d}	0.27± 0.02^{bc}	0.47± 0.03^{a}	0.20± 0.03^{cd}	0.18± 0.009^{cd}	0.30±0. 02^{bc}	0.43±0. 12^{ab}	0.001
Linoleic acid (mg/ml)	0.11± 0.01^{c}	0.34± 0.01^{b}	0.45± 0.02^{a}	0.05± 0.02^{d}	0.05± 0.004^{d}	0.06±0. 003^{d}	0.07±0. 008^{cd}	<0.01

*Means with different superscripts in the same raw are significantly different (p<0.05).

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