Effects of Season and Dietary Cotton Seed Oil Supplementation on the Reproductive Performance of Males And Females Bauscat Rabbits

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Abstract: A 2× 4 factorial design experiment was performed to study the effect of two seasons (Winter and Summer) and four levels of dietary cotton seed oil (0, 30, 60 and 90 g cotton seed diet oil / kg diet) on the performance of males and females of adult Bauscat rabbits. Forty eight of Bauscat does of 6 months of age and twenty four bucks of the same breed (at 7 months of age were used in the present study and randomly divided into 4 treatment groups (6 does and 3 bucks in each group in each season). Results obtained could be summarized as follows: Does performance: The results showed that doe rabbits fed diets contained 90 g cotton seed diet oil / kg diet had significantly ($P< 0.01$) the lower values of feed and water intake than the other groups. Does fed diets contained 90 g cotton seed diet oil / kg diet had significantly ($P \leq 0.05$ and 0.01) higher litter size at weaning, heavier litter weight at 21 days and at weaning and pre-weaning mortality (as number) than the other groups. There were no significant differences in punny weight at different ages and pups gain among the groups studied. Milk yield during the period from birth to 21 days steadily increased with increasing dietary energy level from 0 to 90 g cotton seed oil / kg diet. There were no significant effects due to dietary energy levels on all of blood components studied except for urea which was significantly ($P <0.05$) lower in the group fed diets contained 90 g cotton seed oil / kg diet than the other groups. There were significant ($P \leq 0.05$ and 0.01) differences due to season and dietary oil level interaction effect in litter size at weaning, litter weight at 21 days and at weaning, pre-weaning mortality (as number), feed and water intake and milk yield from birth to 21 days of age. Bucks performance: There are significant ($P \leq 0.05$ and 0.01) differences in all of semen traits, physiological traits and blood components between seasons (winter and summer). Feed and water intake for bucks were significantly ($P <0.05$) decreased when bucks fed diets contained 90 g cotton seed oil / kg diet than the other groups.

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1. Introduction

Productive and reproductive performances are known to be affected by several factors as environmental conditions and managerial factors. Several authors have reported that rabbit breeds are significantly (P≤0.05 and 0.01) differ in their genetic potentialities and productive and reproductive abilities under the Egyptian conditions **(1-6)**. The nutritional factors are one of these important factors which affect the economic intensive rabbit production, under the sub-tropical conditions of Egypt. During summer season, the high temperature affects negatively productive and reproductive performance traits **(7-12)** .Alleviation of heat stress effects by nutritional means can help in keeping the animals in their thermo-neutral state **(13-15)**. Good productive and reproductive performance of buck and doe rabbits was observed when rabbits fed diets of a wide range of energy, i.e. from 2500 to 2900 Kcal DE/kg diet ***(16 and 17)***. Several experiments have included high oil diets for rabbits ***(18)***, and in general, the response is towards better performance of the animals as the level of oil increases ***(19)***. Fats and oils are very energy dense raw materials and their gross energy content is nearly three times as high as that of other feed stuffs ***(20)***. The later author added that the high energy diets (fat-added diets) have to be used with care, whereas when does are not lactating, the increased energy intake would cause excessive fattening with negative consequences on their reproductive capacities. Therefore, fat-added diets have to be limited to lactating does or fed on a restricted basis outside the lactation period. Does lose weight and mobilize body tissue during the lactation period due to energy deficiency and if the quantity and quality of the diet do not control this situation, breeding performances may be seriously impaired ***(21)***. So, the aim of the present work is to evaluating increasing dietary energy level to the rabbit diets (2465, 2650, 2850 and 3050 Kcal DE/ kg diet) on the performance and blood biochemistry of males and females of Bauscat rabbits under the winter and summer conditions of Egypt.

2. Materials and Methods

The present study was carried out at a private farm in Zagazig city, Sharkia governorate, during the summer season period of the year (from June to September) and during the winter season period of the year (from November to April).

A 2× 4 factorial design experiment was performed to study the effect of two seasons (Summer and Winter) and four levels of cotton seed oil (0, 30, 60 and 90 g cotton seed oil / kg diet) on the performance of adult males and females Bauscat rabbits. Forty eight of Bauscat does (24 does of each season) of 6 months of age and twenty four bucks of the same breeds (12 bucks of each season) at 7 months of age were randomly divided into 4 treatment groups (6 does and 3 bucks in each group in each season). All groups were nearly similar in average initial body weights. Averages of ambient temperature and relative humidity values at midday inside the rabbitry building during the experimental period were 27.5^{o}C and 75.3% (in the summer period). Feeding and water were available *ad libitum* at all time.

Four experimental diets were formulated to cover all nutrient requirements for rabbit does and bucks ***(22)***. Table 1 shows the formulation and calculated analysis of the diets used in the present study.

All rabbits were kept under the same managerial, hygienic and environmental conditions and maintained and treated in adherence to accepted standards for the humane treatment of animals. The bucks and does were individually reared in wire cages, in a well ventilated building. Fresh water was available all the time by stainless steel nipples. All cages were equipped with feeders and automatic nipples. During the experimental period, the total artificial light was about 16 hours/day. At mating rabbits were individually transferred to the buck cages for copulation and returned to their own hatches, each doe was palpated at 10 days post-mating to be rebred until pregnancy was establish. Within 12 hours after kindling, litter kits were recorded and weaned at 30 days of age. The traits studied for does were feed and water intake, litter size and weight at each of birth, 21 and 30 days (weaning) of age, litter weight gain, conception rate and mortality rate of pups from birth to 30 days of age were recorded.

Semen was collected from each buck after one week from natural mating the females (which were a subject of a similar study). Two successful ejaculates were collected every two weeks from each buck between 8:00 and 10:00 h by means of an artificial vagina using a female teaser rabbit. The temperature of the inner rubber sleeve of the artificial vagina was adjusted to 41- 43^{o}C. Lubrication of the inner sleeve was performed using white vaseline. Each ejaculate was kept separately for examination. Ejaculate volume was measured in millilitres using a 2 ml calibrated collecting tube, soon after collection. Semen-ejaculate volume was measured to the nearest 0.1 ml. For estimation of mass motility, a drop of freshly collected semen was placed on a slide warmed to 37^{o}C and examined under low magnification by grades from 0 to 5. Advanced motility was examined by placing a small drop of fresh semen on a warm glass slide, diluted with two drops of warm 0.9% saline solution (NaCl) and covered with cover slip. Examination was made under high power magnification (X400). Evaluation of advanced motility was assessed by percentage from 10 to 100%. Percentage of dead spermatozoa was estimated using esion –nigrosin stain technique. Preparation of the stain was carried out by dissolving 10.0 gm nigrosin and 1.67 gm eosin in distilled water up to 100 ml. Seven drops of stain were added to a test tube and warmed to 37^{o}C in a water bath before addition of 0.1 ml semen. One drop of the mixture was removed by Pasteur pipette and placed at the end of a warm slide, then examined under the high power magnification (X400). The live spermatozoa were not stained, while dead spermatozoa were stained pink. Percentage of dead spermatozoa was estimated by counting the number present in 100 sperm in different fields on the slide. Sperm-cell concentration (X10^{6}/ml) was estimated using haemocytometer. Semen was diluted with distilled water plus eosin stain before counting of sperm-cells.

Rectal temperature (^{o}C) and respiration rate (Respirations / minute) were measured in does and bucks once every two weeks at 8.00-10.00 h. Respiration rate was recorded by visual counting the frequency of the flank movement per minute using a hand counter. Internal body temperature was taken by medicine thermometer inserted into the rectum for 2 minutes at depth of 2 cm.

At the end of the experimental period, blood samples were collected from 3 does and 3 bucks of each group. Blood was collected from the marginal ear vein after shaving and cleaning with alcohol in less than 2 minutes into dry clean centrifuge tubes containing some drops of heparin. Plasma was separated by centrifugation at 3000 rpm for 20 minutes and kept in a deep freezer at -20^{o}C until analysis. Total proteins, albumin, total lipids, urea and creatinine concentrations in plasma were estimated using commercial kits (Bio Merieux, France) according to the procedure outlined by the manufacturer. Globulin values were obtained by subtracting the values of albumin from the corresponding values of total proteins. Total milk yield from birth to 21 days of age was calculated according to the following formula ***(23)***: Calculated milk yield from birth to 21 days of lactation (g/doe) = (Litter weight gain during the period from birth to 21 days in kilograms [estimated for the live animals

during the period from birth to 21 days (g) + gain weight (g) of each of its mortals from birth up to the day of its death, during the same period) / 0.56, where 0.56 was the standard value given by Cowie ***(23)*** for the NZW strain depending on the linear relationship between litter weight gain and doe milk consumed.

The data were analyzed statistically according to Snedecor and Cochran ***(24)*** by using SAS computer program as following:

$X_{ijk} = \mu + B_i + O_i + BO_{ij} + E_{ijk}$

Where, X_{ijk} = an observation, μ = Overall mean, B_i = Fixed effect of i^{th} season effect (summer and winter) O_j = Fixed effect of j^{th} dietary energy level (2465, 2650, 2850 and 3050 Kcal Dg/ Kg), and E_{ijk} = Random error.

The differences among means were tested by Duncan's multiple range test ***(25)***.

Table 1. Formulation and calculated analysis of the experimental diets.

Ingredients	Cotton seed oil (g)			
	0	30	60	90
Berseem hay	27.5	29.0	31.0	31.0
Barely	29.0	29.0	29.0	29.0
Wheat bran	24.0	19.6	10.9	7.8
Soybean meal 44%	16.3	18.0	19.7	19.7
Limestone	1.5	1.4	1.0	1.0
Bone meal	1.0	1.3	1.7	1.7
Salt	0.2	0.2	0.2	0.2
Premix*	0.3	0.3	0.3	0.3
DL-Methionine	0.2	0.2	0.2	0.2
Total	100	100	100	100
Calculated analysis**				
Digestible energy (Kcal/kg) DE	2465	2648	2852	3050
Crude protein (CP)	17.7	17.6	17.6	17.6
Crude fiber (CF)	13.2	13.1	13.3	13.3
Ether extract (EE)	2.5	5.4	8.3	8.3
N-Free extract (NFE)	57.2	54.1	50.5	50.5
Ash	9.4	9.8	10.3	10.3

* Each 3 Kg vitamin and mineral mixture contains: Vit. A 12000000 IU, Vit. D_3 2200000 IU, Vit. E 1000 mg, Vit. K 2000 mg, Vit. B_1 1000 mg, Vit. B_2 4000 mg, Vit. B_6 1500 mg, Vit. B_{12} 10 mg, Pantothenic Acid 10000 mg, Niacin 20000 mg, Biotin 50 mg, Folic Acid 1000 mg, Choline chloride 500 gm, Selenium 100 mg, Manganese 5500 mg, Zinc 50000 mg, Iodine 1000 mg and carrier CaCo3 to 3000 gm.
**Calculated according to NRC (1977).

3. Results and Discussion

Does performance:

Season effect:

It is clear from the data found in Tables 2 and 3 that Bauscat doe rabbits had significantly ($P \leq 0.05$ and 0.01) higher values of litter size at weaning, litter weight at 21 days and at weaning, feed intake and milk yield calculated from birth to 21 days of age, and at the same time Bauscat doe rabbits had significantly ($P \leq 0.05$) lower values of pre-weaning mortality in winter than in simmer.

Similar results were obtained by Seleem ***(4)*** who claimed that winter season had significantly ($P \leq 0.01$) higher values of conception rate, litter size at birth and at weaning and bunny weight at birth and at weaning. Results of the present study are in a good agreement with those observed by (***1- 3, 5,6)*** who found a significant ($P \leq 0.05$ and 0.01) differences doe performance due to season effect.

Dietary cotton seed oil levels effect:

Its obvious from Table 2 that group of rabbits fed diets contained 90 g cotton seed oil / kg diet had significantly ($P \leq 0.01$) the lower values of feed and water intake (146.9 g feed/day and 241.9 ml water/day).

Rabbits fed diets contained 90 g cotton seed oil / kg diet had significantly ($P \leq 0.05$ and 0.01) higher litter size at weaning, heavier litter weight at 21 days and at weaining and lesser pre-weaning mortality (as number) than the other groups (Table 2). There were no significant differences in punny weight at different ages and pups gain among the groups studied. Milk yield during the period from birth to 21 days steadily and significantly ($P \leq 0.05$) increased as increasing energy level in the diet from 0 to 90 g cotton seed oil / kg diet (Table 2). Increasing energy intake always leads to a higher milk production and consequently to heavier young during the lactation period ***(20 and 26)***. The same author added that the higher energy intake does not appear to have a positive energy deficit of the doe because fat-added diets primarily stimulate milk production. It could be attributed the positive effect of fat addition to doe diets on the survival of young

rabbits due to greater milk energy resources during the first days of lactation ***(27)***.

Results concerning fertility and prolificacy are controversial. A tendency to positive effects was found when does were submitted to an intensive reproduction rhythm ***(28)***. Adding fat from vegetable sources (total ether extract 99 gm/kg dry matter) or animal sources (total ether extract 117 gm/kg dry matter) did not affect feed intake of rabbit does during lactation ***(29)***. The same author added that adding fat to the diets of rabbit does significantly ($P \leq 0.01$) increased litter size and weight at 21 days of age. Under hot temperature conditions (30^{o}C), there was a positive response to fat-added diets, where litter size was increased, litter weight was heavier at 21 days and at weaning and young survival rate increased (+30%) as found by Fernandez-Carmon ***(30)***. The results of the present work are in agreement with those found by Pascual, Amber and El-aaser ***(27, 31 and 32)*** who reported that the inclusion of high fat levels in the diets of rabbit does significantly ($P \leq 0.05$ and 0.01) improved productive and reproductive performance of rabbit does.

There were no significant differences in rectum temperature and respiration rate among the groups studied.

Regarding blood components, there were no significant effects due to dietary oil levels on all of blood components studied except for urea which was significantly ($P \leq 0.05$) lower in the group fed diets contained 90 g cotton seed oil / kg diet than the other groups studied (Table 4).

Interaction effect between season and dietary energy level:

There were significant ($P \leq 0.05$ and 0.01) differences due to season and energy level effect in litter size at weaning, litter weight at 21 days and at weaning, pre-weaning mortality (as number), feed and water intake and milk yield from birth to 21 days of age (Table 2). On the other hand, the statistical analysis did not show any significant differences in the physiological and blood biochemical parameters (Table 3).

Bucks performance:

Season effect:

It could be seen from results presented in Tables 4 and 5 that there are significant ($P \leq 0.05$ and 0.01) differences in all of semen traits, physiological traits and blood components between winter and summer seasons. On the contrary of the present results, ***(4)*** Seleem indicated that winter season exceeded significantly ($P \leq 0.001$) in the most of semen characteristics studied.

Dietary energy level effect:

Concerning the results found in Table 4, it could be concluded that feed and water intake were significantly ($P \leq 0.05$) decreased when bucks fed diets contained 90 g cotton seed oil /kg diet DE than the other groups.

It could be seen from the results found in Tables 4 and 5 that physical semen characteristics, rectum temperature, respiration rate and blood biochemistry parameters did not significantly affect by increasing dietary energy level**.** El-aaser ***(32)*** claimed that bucks fed diets of high energy (90 g cotton seed oil /kg diet) had insignificantly higher values of semen volume, and significantly ($P \leq 0.01$) higher values of semen sperm cell concentration (x 10^{6}/ml), advanced motility (%) and lesser ($P \leq 0.01$) values of dead spermatozoa (%) than the control diet (30 g cotton seed oil /kg diet). Linoleic and Oleic diets had a negative impact ($P \leq 0.05$) on the total sperm motility and viability after a 6 week period of dietary supplementation ***(33)***.

Similar results were obtained by ***(34)*** who found that supplementation of 1% acidulated palm oil insignificantly improved, in general, the quality of bucks' semen. Sunflower oil supplementation to the rabbit diets did not alter blood lipids ***(35)***.

Interaction between season and dietary energy level effect:

It is clearly that there are significant ($P \leq 0.05$) differences in all of buck traits studied due to interaction between season and dietary energy level (Tables 4 and 5).

From the results of the present work, it could be concluded that increasing dietary oil up to 90 g cotton seed oil / kg diet show some potential to improve performance of the Buscat rabbits at high and mild environmental temperatures, but need more investigations especially with protein level and quality of fat enriched diets with the different seasons.

Table 1. Feed intake, water intake, rectal temperature and respiration rate of Bauscat female rabbits as affected by dietary supplementation with cotton seed oil, under the summer conditions condition of Egypt.

Items	Control 0 % / kg diet	Cotton seed oil 3 % / kg diet	Cotton seed oil 6 % / kg diet	Cotton seed oil 9 % / kg diet	Sig.
Feed intake (g /day)	191.6± 3.7 [c]	177.5± 3.2 [b]	163.2± 2.8 [b]	141.1± 2.7 [a]	**
Water intake (ml /day)	369.1±9.6 [c]	280.2±12.1 [b]	260.9±10.5 [a]	251.2±11.9 [a]	**
Rectum temperature(^{o}C)	39.1±0.09	39.3±0.09	39.0±0.08	39. 1±0.09	NS
Respiration rate (Respirations/minute)	118.6±1.2	113.4±1.1	116.9±1.1	119.9±1.0	NS

Means bearing different letters in the same column within each classification, differ significantly ($P \leq 0.05$).
** = P<0.01 and NS = Not significant

Table 2. Bauscat female rabbits traits as affected by dietary supplementation with cotton seed oil, under the summer conditions condition of Egypt.

Items	Control 0 % / kg diet	Cotton seed oil 3 % / kg diet	Cotton seed oil 6 % / kg diet	Cotton seed oil 9 % / kg diet	Sig.
Litter size at					
Birth	3.7±0.32	4.0±0.39	4.1±0.41	4.0±0.30	
21 days	3.0±0.30	3.3±0.33	3.4±0.35	3.6±0.32	
Weaning	2.3±0.47 [b]	2.8±0.42 [a]	3.1±0.50 [a]	3.2±0.53 [a]	**NS**
Litter weight at					**NS**
Birth	140.88±3.57	140.6±3.02	159.58±3.95	158.70±3.12	*
21 days	554.82±6.42 [b]	619.09±6.8 [ab]	649.2±7.1 [a]	730.63±7.9 [a]	
Weaning	469.18±7.01 [b]	660.06±6.8 [a]	709.46±8.9 [a]	792.79±8.7 [a]	**NS**
Pups gain	305.92±4.05	326.50±4.18	331.6±4.37	334.62±4.02	*
Pre weaning mortality (as number)	1.5 ±0.63 [a]	1.2±0.69 [a]	1.2±0.55 [b]	0.9±0.47 [b]	**
Conception rate%	37.1	38.3	38.7	40.6	NS
Number of parity during experimental period	4.2	4.2	4.5	4.6	*
Feed cost (LE per doe)	66.07	70.61	74.66	74.04	
Return from all weaning rabbits per doe (LE)	70.98	98.28	111.15	125.58	
Margin (LE per doe)	4.91	27.67	36.49	51.54	

Means bearing different letters in the same column within each classification, differ significantly (P≤0.05).
** = P<0.01, * = P<0.05 and NS = Not significant
Price: Experimental diet = 1.9 LE per kg for control, 2.2 LE per kg for 3% cotton seed oil, 2.5 LE per kg for 6% cotton and 2.8 LE per kg for 9% cotton seed oil – weaning rabbit = 13 – Margin per doe =Return from all weaning rabbits per doe – feed cost . other doe costs were assumed constant.

Table 3. Effect of cotton seed oil supplementation on blood chemicals of Bauscat female rabbits, under summer Egyptian conditions (x'± SE).

Items	Control 0 % / kg diet	Cotton seed oil 3 % / kg diet	Cotton seed oil 6 % / kg diet	Cotton seed oil 9 % / kg diet	Sig.
Total protein (g/dl)	7. 4±0.53	7.1±0.59	6.9±0.46	7.1±0.61	**N.S**
Albumin (g/dl)	3.9±0.26	4.08±0.29	3.7±0.31	4.1±0.24	**NS**
Golbulin (g/dl)	3.5±0.122	3.02±0.19	3.2±0.14	3.0±0.28	**N.S**
Total lipids (mg/100)	373.77±29.14	381.14±27.01	386.4±22.00	390.5±19.54	**N.S**
Urea (mg/dl)	50.24±3.11 [a]	49.55±4.85 [a]	44.9±2.17 [ab]	37.2±1.99 [b]	*
Creatinine (mg/dl)	1.21±0.09	1.14±0.09	1.09±0.08	1.12±0.09	**NS**

N S = not significant and * (p< 0.05). Means in the same row bearing different letters, differ significantly (p< 0.05).

Table 4. Feed intake, water intake, rectum temperature and respiration rate of Bauscat male rabbits as affected by dietary supplementation with cotton seed oil,under the summer conditions of Egypt.

Items	Control 0 % / kg diet	Cotton seed oil 3 % / kg diet	Cotton seed oil 6 % / kg diet	Cotton seed oil 9 % / kg diet	Sig.
Feed intake (g /day)	88.2± 2.6 [a]	80.1± 2.7 [a b]	77.2± 2.3 [a]	72.8± 1. 9 [a]	*
Water intake (ml /day)	153.21±8.5 [a]	142.31±7.9 [a b]	141.9±7.1 [b]	132.72±8.1 [b]	*
Rectum temperature(^{o}C)	39.9±0.08	39.9±0.07	39.5±0.09	39.4±0.09	NS
Respiration rate (Respirations/minute)	112.1±1.14	109.2±1.21	112.7±1.19	114.2±1.21	NS

Means bearing different letters in the same column within each classification, differ significantly (P≤0.05). NS = Not significant

Table 5. Bauscat male rabbits semen characteristics as affected by dietary supplementation with cotton seed oil , under the summer conditions condition of Egypt.

Items	Control 0 % / kg diet	Cotton seed oil 3 % / kg diet	Cotton seed oil 6 % / kg diet	Cotton seed oil 9 % / kg diet	Sig.
Volume (ml)	0.34± 0.69	0.37±0.71	0.39±0.77	0.40± 0. 65	NS
Mass motility(u)	1..97± 0.72	1.99±0.68	2.0±0.69	2.2±0. 74	**NS**
Advanced motility(%))	26.1± 4.2	27.05± 3.9	30.2± 3.5	31.7± 3.1	**NS**
Dead spermatozoa(%)	20.9± 3.7	20.2± 3.3	18.1± 3.1	17.4± 3.9	**NS**
Sperm concentration (10^{6} x ml)	259.46± 34.2	262.71± 35.8	265.98± 39.6	278.11± 40.9	**NS**

Means bearing different letters in the same column within each classification, differ significantly (P≤0.05).
NS = Not significant

Table (4):- Effect of cotton seed oil supplementation on blood chemicals and blood picture of Bauscat male rabbits, under summer Egyptian conditions (x'± SE).

Items	Control 0 % / kg diet	Cotton seed oil 3 % / kg diet	Cotton seed oil 6 % / kg diet	Cotton seed oil 9 % / kg diet	Sig.
Total protein (g/dl)	7. 0±0.98	6.61±0.76	6.3±0.49	6.8±0.87	**N.S**
Albumin (g/dl)	3.9±0.18	3.4±0.16	3.2±0.13	3.2±0.19	**N.S**
Golbulin (g/dl)	3.1±0.22	3.21±0.19	3.1±0.19	3.6±0.24	**N.S**
Total lipids (mg/100)	360.01±24.52	358.33±25.29	368.02±37.11	379.9±36.19	**N.S**
Urea (mg/dl)	40.1±1.72	41.9±1.99	42.3±1.95	41.7±1.68	**N.S**
Creatinine (mg/dl)	1.21±0.06	1.23±0.07	1.26±0.08	1.20±0.08	**N.S**

N S = not significant . Means in the same row bearing different letters, differ significantly ($p< 0.05$).

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