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Can feed supplementation of the refined vegetable oils enhance the seminal quality of rabbit bucks (*Oryctolagus cuniculus*)?

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Abstract

Exploring new feeding strategies are a necessary aspect for improving the reproductive performance in rabbits. Twenty healthy rabbit bucks with a mean live body weight of 1.01 kg (SD = 0.12) and age of 6 months old were used for a period of 17 weeks to examine the influence of feeding soybean and sunflower oils on their reproductive performance. Rabbits were randomly assigned into 4 groups (5 bucks/group), where bucks in the 1st group, served as a control, were fed for 14 weeks on a standard ration without any oil supplementation, while bucks in the 2nd, 3rd, and 4th groups received -on the basis of the inclusion rate- a ration supplemented with 3% soybean oil, 3% sunflower oil, and 1.5% soybean oil plus 1.5% sunflower oil, respectively. Climatic, bio-physiological, blood and seminal measurements were all been determined. The obtained results suggested that offering rations supplemented with soybean and/or sunflower oils at the level of 3% of DM to rabbit bucks had no impacts on their health status, based on the findings that feed conversion ratio, blood hematology as well as liver and kidney functions were all not altered; thereby, indicating that the refined vegetable oils can be safely supplemented into rabbits rations. Most importantly, the collected evidences proposed that supplementing vegetable oil-enriched rations to rabbit bucks during their adulthood may demonstrate subsequent positive influences on their reproductive characteristics as early as the 3rd/4th week after feeding on such oils. This was generally manifested by the higher ($P < 0.05$) sperm concentration, total sperm output, percentage of motile sperms, as well as the lower ($P < 0.05$) percentages of dead and altered acrosomal sperms that observed in bucks compared to their control twins. Based on the obtained results herein, feeding rations supplemented with soybean and/or sunflower oils at the level of 3% of DM to rabbit bucks during their adulthood would produced an acceptable semen quality compared to the control bucks. Research dealing with such aspect may improve our understanding of the nutritional requirements and production of rabbits. However, further researches are definitely imperative because of the number of bucks per group was considerably low in the current experiment.

Keywords: physiology, rabbits, reproduction, semen, soybean oil, sunflower oil.

Introduction

Feeding ration plays an important role within the intensive rabbit production system, where it should encompasses all the essential nutrients ranging from the carbohydrates to minerals. Dietary lipids, however, constitute the main nutrient as they have the most opulent caloric value among all nutrients (McDonald *et al.,* 2011). Several studies on lipid metabolism have shown that mammals, including rabbits, lack the ability to synthesize some of the essential lipids such as omega-3 (ω_3), omega-6 (ω_6), and omega-9 (ω_9; Cheeke and Cunha, 1987; Calvani and Benatti, 2003; Vrablik and Watts, 2013); and consequently they must be provided with the diet.

Our search in the literature illustrates that inclusion of fats had increased the energy density of the ration (Coppock and Wilks, 1991; Caputo and Mattes, 1992; Thomas *et al.,* 1992), improved ration palatability (Fernández-Carmona *et al.,* 2000), enhanced the absorption of fat-soluble vitamins (Cobos *et al.,* 1993; Baião and Lara, 2005), and boosted the dietary digestibility coefficients and feed conversion efficiency (Santoma *et al.,* 1987); all of which led to promote an optimum growth and subsequently conferred several economical advantages (Al-Athari and Watkins, 1988; Ayyat, 1991; Fernandez and Fraga, 1992).

Despite the fact that there are a wide variety of fats that can be supplemented to the rations, plant-based oils primarily extracted from seeds such as palm, cottonseed, soybean, sunflower, rapeseed, coconut, peanut and olives; had manifested several advantages over other sources of fat like animal and synthetic sources. One of the substantial advantages is that these vegetable oils are considered a rich source of essentially polyunsaturated fatty acids (PUFA; Baldini *et al.,* 2000; Calvani and Benatti, 2003). In fact, supplying refined vegetable oils to mammalian and birds rations has been observed to have positive effects on growth rate, carcass traits and meat composition (Santoma *et al.,* 1987; Beynen, 1988; Ayyat, 1991; Keteslegers *et al*., 1995; Soliman *et al*., 1999; Whitney *et al.,* 2000; Vieira *et al.,* 2006). However, it is not clear what are the subsequent effects of supplementing such oils on the reproductive performance (i.e. semen quality, fertility and prolificacy) of both mammals and birds.

In fact, the reviewed data and information by various researchers showed an improvement of seminal characteristics in males as well an enhancement of the

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fertility and prolificacy in female animals when fed on ration contained vegetable oils, in one the hand as have been found in rabbits (Christ *et al.,* 1996), horses (Squires, 2006), rats (Naseem *et al.,* 2007), boars (Estienne *et al.,* 2008), dogs (Rocha *et al.,* 2009), turkeys (Zaniboni and Cerolini, 2009), quails (Al-Daraji *et al.,* 2010), and ducks (Ghonim *et al.,* 2010). On the other hand, it is well established that essential-fatty-acid deficiency leads to degeneration of the testis with concomitant infertility (Ahluwalia *et al.,* 1967; Marzouki and Coniglio, 1982; Wathes *et al.,* 2007), and leads to prolonged diestrus, abortion, or giving birth to stillborn fetuses (Menon *et al.,* 1981; Parlanti and Orellana, 1985). Meanwhile, Pascual *et al*. (1999) did not encounter any differences in the prolificacy of does rabbits supplemented with soybean oil ration (at 99 to 117 g per kg DM) compared to control diet (at 26 g per kg DM), as well Kelso *et al*. (1997) who observed no differences in sperm motility during a 49-week experiment when fed a rich ω_3 or ω_6 diets to turkeys, while Yaakub *et al*. (2009) found out that spermatogenesis was altered in rams fed on palm kernel cake-based ration.

This prompts us to raise the question; can feed supplementation of the refined vegetable oils enhance the seminal quality of rabbit bucks? The impact of combined dietary inclusion of soybean (*Glycine max*) and sunflower (*Helianthus annuus*) oils on the reproductive capacities of rabbits has never been investigated. Thus, the present experiment was designed to identify the subsequent influences of supplementing soybean oil- and sunflower oil-enriched rations on the seminal characteristics of rabbit bucks. It was hypothesized that such combined inclusion of vegetable oil would enhance the reproductive performance of rabbit bucks without affecting their health.

Materials and Methods

Location

The present experiment was carried out at the Department of Environmental Studies, Institute of Graduate Studies and Research, Alexandria University, Alexandria, Egypt (+31 deg 12' N +29 deg 54' E).

Animals and management

Twenty healthy rabbit bucks with a mean live body weight of 1.01 kg (SD = 0.12) and age of 6 months old were used for the experiment. They were housed individually in universal galvanized wire cages, where feed and water were offered *ad libitum*. Ventilator fans were timed to operate for 15 min at 2 h intervals. Using a hydrothermograph, climatic data recorded throughout the experiment (i.e. ambient temperature of 23.82 ± 2.13˚C and relative humidity of 53.01 ± 5.10%) indicates a standard laboratory environment with no stressful impacts.

The ration, used in the present experiment, was a commercial pre-formulated pelleted total mixed ration (Nubaria Station, Eygpt). According to the manufacturer's specifications, the TMR consisted of barley, dry clover, soybean, molasses, maize, bran, limestone, methionine, and mineral salt; as well contained on DM basis 18% crude protein, 2% ether extract, 14% crude fiber, and 2.6 Mcal/kg digestible energy.

Experimental design

Current experiment was divided into two stages; preliminary and experimental phases. Bucks were given a preliminary phase of three weeks before commencing the experiment. This phase served as an acclimation phase during which bucks were accustomed to the housing and measuring equipments, prophylactically vaccinated against enterotoxaemia, pasteurellosis, and hemorrhagic (calicivirus) disease, as well as trained to use an artificial vagina for collecting ejaculates.

At the 1st day of the experimental phase (i.e. 14 weeks), bucks were randomly segregated into 4 groups (5 rabbits/group). The 1st group, served as a control, was fed on commercial TMR without any oil supplementation. Meanwhile, the 2nd, 3rd, and 4th groups (designated as T1, T2, and T3, respectively) received -on the basis of the inclusion rate- a ration supplemented with 3% soybean oil, 3% sunflower oil, and 1.5% soybean oil plus 1.5% sunflower oil, respectively. Experimental measurements -mentioned later- were determined during this phase. It is worthwhile to mention that all procedures described herein were approved by the Committee of Research Ethics at the Alexandria University.

Experimental measurements

Biophysiology

Feed intake of each buck was measured on a daily basis by subtracting the refusal weight from the total weight of offered feed using a standard balanced measure to the nearest 10 g. Meanwhile, individual body weights were recorded weekly, at the morning before experimental rations were introduced, utilizing a standard balanced measure to the nearest 100 g. Thereafter, body weight gains as well as feed conversion ratios were both calculated.

Blood collection and analysis

In the morning before feeding, blood samples (3-4 ml) were biweekly collected from the marginal auricular vein of each animal into heparinized tubes, placed inside an ice box, and immediately transferred to the laboratory to be analyzed.

Whole blood samples were analyzed shortly after collection for hematological parameters. Red blood cells (RBCs, $\times 10^6/mm^3$) and white blood cells (WBCs, $\times 10^6/mm^3$) were counted using the hemocytometer method, packed cell volume (PCV, %) was measured using the microhematocrit method, while hemoglobin concentration (Hb, mg/dl) was spectrophotometrically determined using a colorimetric assay (Diamond

Diagnostics, Egypt).

Plasma samples were, thereafter, obtained by centrifugation of blood at 1500 g for 10 min, and stored at -20°C until analysis. Biochemical parameters of blood plasma were spectrophotometrically measured using the Automatic Biochemical Analyzer, Microlab 300 (Merck, Germany) with colorimetric kits from same manufacturer. The following parameters; total protein (mg/dl), glucose (mg/dl), cholesterol (mg/dl), triglyceride (mg/dl), aspartate aminotransferase (AST, IU/L), alanine aminotransferase (ALT, IU/L), urea (mg/dl), and creatinine(mg/dl), were all quantified in the plasma samples.

Semen collection and analyses

Semen samples were collected once weekly (with 1 ejaculate/buck) using the artificial vagina method. The ejaculated semen was collected in a rubber funnel terminated at a transparent graduated tube measure to the nearest 0.10 ml. Immediately after collection, the ejaculates were immersed in a water path (37 ± 2°C) and transported to the laboratory for assessment. Semen analysis was performed within approximately 30 min of collection; thus, measurement does not reflect the initial motility. Sperm cell concentrations ($x10^6$/ml) were estimated using the hemocytometer method according to Smith and Mayer (1955). The total sperm output ($x10^6$) per ejaculate was, then, calculated by multiplying the obtained semen volume (ml) with sperms concentration ($x10^6$/ml).

Moreover, percentages of motile, live, dead, abnormal, and altered acrosomal spermatozoa for each sample were assessed using eosin-nigrosin staining technique. Briefly, a drop of stain was mixed with a drop of pure semen and extended on the slide. Then, 100 spermatozoa were counted using a light microscope equipped with warm stage at 400-1000X magnification. The unstained spermatozoa with a normal appearance were determined as live spermatozoa, the unstained spermatozoa with an abnormal appearance were classified as abnormal spermatozoa, while stained spermatozoa were designated as dead spermatozoa.

Afterward, seminal plasmas were separated from ejaculates by centrifugation at 1500 g for 10 min. Determination of seminal fructose (mg/ml) was colorimetrically assayed using a commercial reagent kit (Diamond Diagnostics, Egypt) according to the method of Mann (1948). It is worth to mention that the same technician oversaw all of the semen collection and analysis processes.

Statistical analysis

For better clarification, the collected data during the 14 weeks of the experimental phase were divided in to 7 periods (i.e. 2 weeks/period). The randomized complete block design (RCBD) is the ideal model to use for data consisting of serial measurements. Therefore, the recorded and estimated data during the experimental phase were analyzed using the PROC MIXED procedure of statistical analysis system (SAS Inst., Inc., Cary, NC), where the time as a repeated measure (i.e. the repeated collection of ejaculate throughout the 7 periods of the experimental phase) was included in the model together with treatment and their interactions as fixed effects, whereas the animal was included as a random effect. The PROC MEANS procedure was also used to obtain the descriptive statistics of all parameters. Thereafter, data were subjected to ANOVA using $\alpha = 0.05$. Means showing significant differences in ANOVA were tested using the PDIFF option. The probability value, which denotes statistical significance, was set at $P < 0.05$. Means and their pooled SEM are presented, unless otherwise indicated.

Results

The influence on health indicators

Table 1 shows the changes of biophysiological as well as blood hematological and biochemical parameters of rabbit bucks fed rations supplemented with different levels of refined vegetable oils. The obtained data revealed that supplementing rations with refined vegetable oils showed ($P < 0.05$) changes in the biopysiological responses of rabbit bucks (Table 1). In fact, it was clear, in comparison with the control group, that daily feed intake was increased ($P < 0.05$) in bucks receiving a ration supplemented with combined soybean and sunflower oils (T3), but it decreased ($P < 0.05$) in bucks fed a ration supplemented with soybean oil alone (T1) and further decreased ($P < 0.05$) in bucks fed on sunflower oil alone (T2; Table 1). Meanwhile, body weight gain of bucks belonged to the T1 and T3 groups showed ($P < 0.05$) increases, whereas no effects ($P < 0.05$) was observed on the bucks of the T2 group, compared to bucks in the control group (Table 1). Nevertheless, feed conversion ratios calculated for all groups exhibited -to some extent- comparable figures.

Notably, the overall means of all hematological and biochemical parameters (i.e. RBCs, WBCs, Hb, PCV, total protein, glucose, cholesterol, triglyceride, AST, ALT, urea, and creatinine levels) measured throughout the experiment were, in a distinguish way, uninfluenced ($P > 0.05$) in bucks fed rations treated with soybean and sunflower oils (Table 1).

The influence on seminal characteristics

Generally speaking, positive responses ($P < 0.05$) in the physical and biochemical characteristics of semen ejaculates were seen as early as the 3rd - 4th week after feeding rabbit bucks on rations supplemented with different levels of refined vegetable oils compared to bucks fed on the control ration (Tables 2, 3, and 4, as well as Fig. 1). In fact, the overall means of sperm concentration and percentages of motile sperm were higher ($P < 0.05$) while overall means of altered acrosomal sperm percentages were lower ($P < 0.05$) in bucks belonged to the 2nd, 3rd, 4th groups, compared to bucks in the control group as early as the 2nd period (3rd/4th week) after feeding on such oils. Moreover, dietary treatments on refined vegetable oils decreased

progressively the overall means of dead sperm percentages and total sperm output from the 3rd period (5th/6th week) and 5th period (10th/11th week) after feeding, respectively (Tables 3 and 4, as well as Fig. 1).

Meanwhile, the overall mean of the ejaculate volume did not differ ($P > 0.05$) between groups (Table 2). Measuring the ejaculated volume was performed using a transparent graduated tube measure to the nearest 0.10 ml. Such low accuracy (i.e. using 0.10 ml for a total volume of less than 1 ml) could be the reason of the lack of significancy of the supplementation. Moreover, there was no subsequent effect ($P > 0.05$) of dietary treatments on the concentration of seminal fructose throughout the experiment (Table 2 and Fig. 1).

Table 1. Health indicators of rabbit bucks fed rations supplemented with different levels of refined vegetable oils (n = 5 bucks per treatment).

Parameters[1]	Feeding treatments[2]				SEM[3]
	Control	T1	T2	T3	
Biophysiology					
DFI (g)	214.37^{b}	192.41^{c}	171.59^{d}	234.40^{a}	6.03
BWG (kg)	1.47^{c}	1.81^{a}	1.51^{c}	1.67^{b}	0.04
FCR[4]	0.15^{a}	0.11^{b}	0.12^{b}	0.14^{a}	0.01
Haematology					
RBCs ($\times 10^6$/mm^3)	11.64	11.83	12.84	11.12	0.64
WBCs ($\times 10^6$/mm^3)	4.08	4.56	4.34	4.44	0.65
Hb (mg/dl)	8.99	9.28	10.25	10.07	0.70
PCV (%)	42.66	44.49	45.32	42.39	2.02
Biochemistry					
Total protein (mg/dl)	3.86	3.74	3.76	3.62	0.22
Glucose (mg/dl)	88.71	83.16	87.30	89.94	7.46
Cholesterol (mg/dl)	95.95	103.46	100.19	101.99	4.08
Triglyceride (mg/dl)	111.48	111.41	108.95	93.29	6.31
AST (IU/L)	73.57	82.43	87.94	82.74	7.69
ALT (IU/L)	25.39	26.77	24.95	20.75	2.32
Urea (mg/dl)	38.57	37.36	42.67	41.70	1.77
Creatinine (mg/dl)	1.04	0.94	1.04	0.97	0.15

[1]DFI: daily feed intake; BWG: body weight gain; FCR: feed conversion ratio; RBCs: red blood cells; WBCs: white blood cells; Hb: hemoglobin; PCV: packed cell volume, AST: aspartate aminotransferase, and ALT: alanine aminotransferase. [2]Throughout the experimental phase, rabbit bucks were fed on control ration (Control), ration contained 3% soybean oil (T1), ration contained 3% sunflower oil (T2), and ration contained 1.5% soybean oil plus 1.5% sunflower oil (T3). [3]Due to the effect of feeding treatment alone. [4]feed conversion ratio was calculated by the division of daily feed intake by the average daily gain. [a-c]Means within the same row bearing different superscripts are significantly different at $P < 0.05$.

Table 2. Physical and biochemical characteristics of semen ejaculates collected throughout the whole experiment (14 weeks) from rabbit bucks fed rations supplemented with different levels of refined vegetable oils (n = 5 bucks per treatment).

Parameters	Treatments[1]				SEM[2]
	C	T1	T2	T3	
Physical characteristic					
Ejaculate volume (ml)	0.44	0.36	0.49	0.43	0.06
Sperms concentration (x10^6/ml)	17.74^{b}	62.86^{a}	60.21^{a}	50.21^{a}	6.28
Total sperm output (x10^6)	7.69^{c}	25.13^{ab}	30.65^{a}	19.06^{b}	2.81
Sperms motility (%)	23.60^{b}	49.57^{a}	56.86^{a}	47.21^{a}	7.09
Abnormal sperms (%)	16.31^{bc}	22.52^{a}	12.84^{c}	17.76^{b}	1.55
Dead sperms (%)	17.58^{a}	12.20^{b}	8.13^{b}	12.45^{b}	1.83
Altered acrosomes (%)	11.09^{a}	5.70^{b}	5.89^{b}	6.24^{b}	0.56
Biochemical characteristic					
Fructose (mg/ml)	1.24	1.19	1.30	1.20	0.13

[1]Throughout the experimental phase, rabbit bucks were fed on control ration (Control), ration contained 3% soybean oil (T1), ration contained 3% sunflower oil (T2), and ration contained 1.5% soybean oil plus 1.5% sunflower oil (T3). [2]Due to the effect of feeding treatment alone. [a-b]Means within the same row bearing different superscripts are significantly different at $P < 0.05$.

Table 3. Physical characteristics of semen ejaculates collected during the first 4 periods (i.e. period no. 1, 2, 3, and 4; 2 weeks/period) from rabbit bucks fed rations supplemented with different levels of refined vegetable oils (n = 5 bucks per treatment).

Parameters	Treatments[1]				SEM[2]
	C	T1	T2	T3	
Period no. 1					
Sperms concentration (x10^6/ml)	18.50	28.75	27.75	22.25	5.60
Total sperm output (x10^6)	8.30	5.75	5.55	7.45	7.43
Sperms motility (%)	12.00	19.00	21.00	18.00	3.65
Abnormal sperms (%)	20.50^b	25.00^a	21.40^b	25.90^a	1.32
Dead sperms (%)	20.40^a	21.40^a	16.30^b	23.30^a	1.25
Altered acrosomes (%)	8.90	7.60	9.10	9.40	1.08
Period no. 2					
Sperms concentration (x10^6/ml)	17.36^b	44.50^a	43.50^a	36.50^a	5.60
Total sperm output (x10^6)	6.04	8.90	17.40	10.50	7.43
Sperms motility (%)	19.00^b	29.00^a	34.00^a	18.00^b	3.65
Abnormal sperms (%)	15.00^c	24.30^a	19.10^b	22.60^{ab}	1.32
Dead sperms (%)	18.55^a	18.70^a	10.70^b	15.23^a	1.25
Altered acrosomes (%)	11.30^a	6.80^b	6.00^b	7.20^b	1.08
Period no. 3					
Sperms concentration (x10^6/ml)	16.48^c	66.50^a	53.50^b	42.00^b	5.60
Total sperm output (x10^6)	7.98	13.30	26.75	11.55	7.43
Sperms motility (%)	29.00^b	47.00^a	52.00^a	41.00^a	3.65
Abnormal sperms (%)	12.80^c	23.50^a	13.50^c	19.00^b	1.32
Dead sperms (%)	19.10^a	13.30^b	7.80^c	16.40^{ab}	1.25
Altered acrosomes (%)	13.10^a	6.30^b	4.70^b	6.30^b	1.08
Period no. 4					
Sperms concentration (x10^6/mL)	17.25^b	68.50^a	65.75^a	52.50^a	5.60
Total sperm output (x10^6)	9.38	17.18	27.30	14.60	7.43
Sperms motility (%)	28.00^b	55.00^a	56.00^a	53.50^a	3.65
Abnormal sperms (%)	13.70^c	22.06^a	10.90^c	16.10^b	1.32
Dead sperms (%)	14.20^a	9.90^b	6.00^b	9.70^b	1.25
Altered acrosomes (%)	11.60^a	7.00^b	5.30^b	5.30^b	1.08

[1]Throughout the experimental phase, rabbit bucks were fed on control ration (control), ration contained 3% soybean oil (T1), ration contained 3% sunflower oil (T2), and ration contained 1.5% soybean oil plus 1.5% sunflower oil (T3). [2]Treatment x time interaction. [a-d]Means within the same row bearing different superscripts are significantly different at $P < 0.05$.

Table 4**.** Physical characteristics of semen ejaculates collected during the last 3 periods (i.e. period no. 5, 6, and 7 2 weeks/period) from rabbit bucks fed rations supplemented with different levels of refined vegetable oils (n = 5 bucks per treatment).

Parameters	Treatments[1]				SEM[2]
	C	T1	T2	T3	
Period no. 5					
Sperms concentration (x10^6/ml)	16.72^c	70.75^{ab}	74.50^a	57.75^b	5.60
Total sperm output (x10^6)	5.85^b	24.78^a	44.70^a	17.50^{ab}	7.43
Sperms motility (%)	25.80^b	58.00^a	64.00^a	58.00^a	3.65
Abnormal sperms (%)	16.30^b	21.96^a	9.60^c	14.70^b	1.32
Dead sperms (%)	17.29^a	8.20^b	5.70^b	8.40^b	1.25
Altered acrosomes (%)	11.20^a	5.60^b	4.70^b	5.70^b	1.08
Period no. 6					
Sperms concentration (x10^6/ml)	21.83^c	78.00^a	75.75^a	62.50^b	5.60
Total sperm output (x10^6)	8.73^b	39.60^a	45.45^a	25.00^{ab}	7.43
Sperms motility (%)	23.60^c	63.00^b	79.00^a	61.00^b	3.65
Abnormal sperms (%)	15.70^b	21.16^a	9.00^c	13.60^b	1.32
Dead sperms (%)	14.90^a	7.50^b	5.40^b	7.70^b	1.25
Altered acrosomes (%)	10.00^a	3.60^b	4.60^b	4.80^b	1.08
Period no. 7					
Sperms concentration (x10^6/ml)	18.00^b	83.00^a	80.75^a	78.00^a	5.60
Total sperm output (x10^6)	7.56^b	66.40^a	48.45^a	46.80^a	7.43
Sperms motility (%)	27.80^b	76.00^a	82.00^a	81.00^a	3.65
Abnormal sperms (%)	20.20^a	19.66^a	6.40^c	12.40^b	1.32
Dead sperms (%)	18.60^a	6.40^b	5.00^b	6.40^b	1.25
Altered acrosomes (%)	11.50^a	4.00^b	6.80^b	5.00^b	1.08

[1]Throughout the experimental phase, rabbit bucks were fed on control ration (control), ration contained 3% soybean oil (T1), ration contained 3% sunflower oil (T2), and ration contained 1.5% soybean oil plus 1.5% sunflower oil (T3). [2]Treatment x time interaction. [a-d]Means within the same row bearing different superscripts are significantly different at $P < 0.05$.

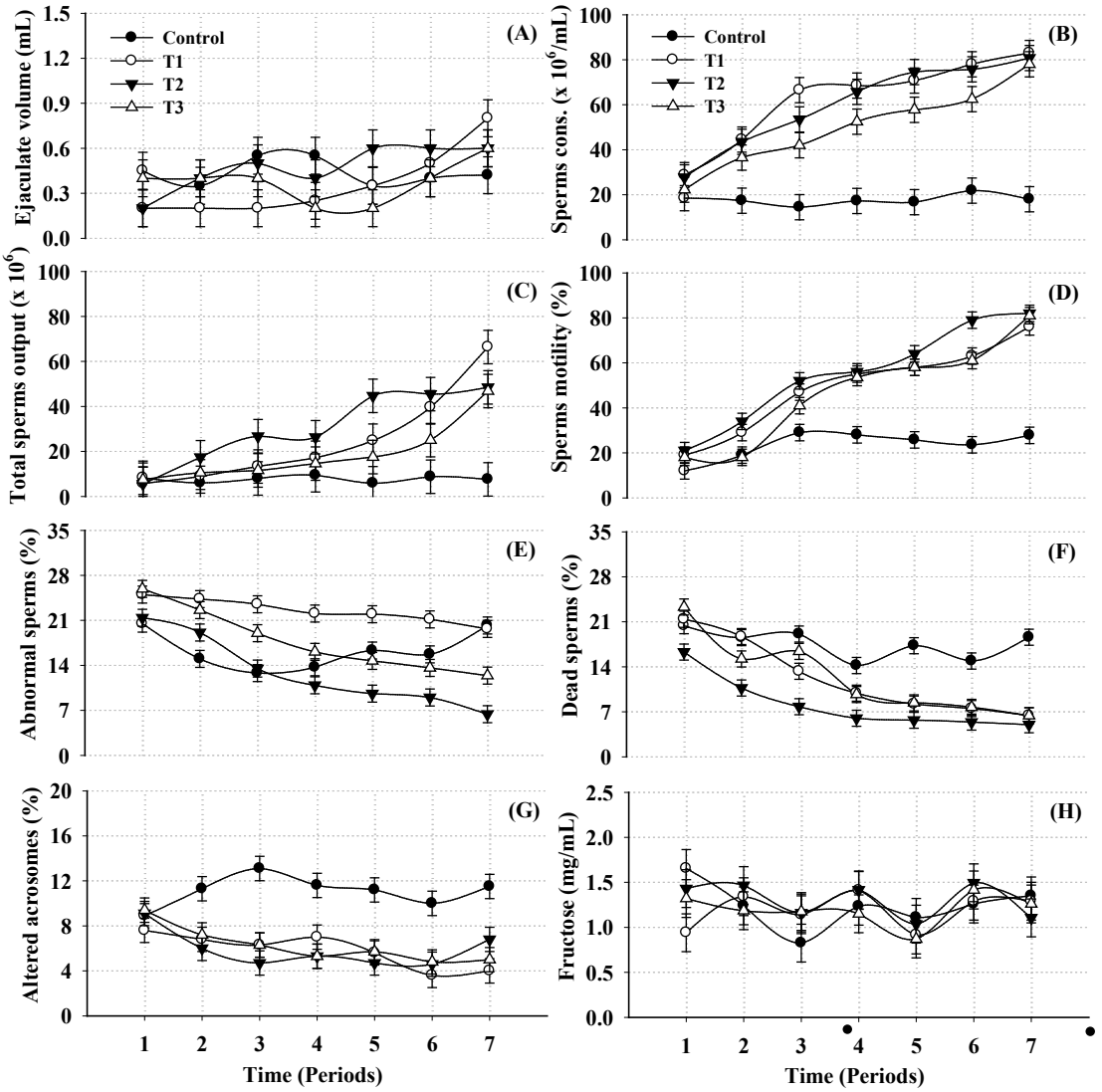

Figure 1. Depiction of least-squares means for physical and chemical characteristics of semen ejaculates collected during seven periods (2 weeks/period) from rabbit bucks fed rations supplemented with different levels of refined vegetable oils (n = 5 bucks per treatment). For all panels, C = control ration, T1 = ration contained 3% soybean oil, T2 = ration contained 3% sunflower oil, and T3 = ration contained 1.5% soybean oil plus 1.5% sunflower oil. Data are presented as mean ± SEM. For statistical differences between treatments within each period, refer to Table 3 and 4.

Discussion

Adequate nutrition is critical for successful reproductive functions, which is considered the main factor that assures high productivity of rabbit farms. Since Burr and Burr (1930) had demonstrated the essentiality of ω_3, ω_6, and ω_9 fatty-acid on the growth and reproduction performance in animals, a wide range of physiological functions has been shown to be affected by essential fatty acid deficiency in mammals. Impairment of reproductive functions is one of the earlier symptoms of the lack of essential fatty acids in rats (Menon *et al*.**,** 1981; Ravel *et al.,* 1985; Guesnet *et al.,* 1986) and humans (Wathes *et al.,* 2007). Accordingly, the present experiment was conducted to determine whether supplementing different types and levels of refined vegetable oils to the ration would enhance the seminal characteristics in rabbit bucks. To our knowledge, this is the first report on the reproductive responses of bucks to a combined inclusion of dietary soybean and sunflower oils supplementation.

Feeding rations supplemented with soybean and/or sunflower oils at the level of 3% of DM to rabbit bucks during their adulthood period had no impacts on their health status, based on the findings that blood hematology as well as liver and kidney functions were not altered; thereby, suggesting that the refined vegetable oils can be safely supplemented into rabbits rations. Despite the fact that daily feed intake and body weight gain was increased, supplementing soybean oil and sunflower oil-enriched rations to bucks failed to demonstrate any benefits to their growth performance compared to the control bucks. In fact, it was clear that

the calculated feed conversion ratios for all groups were exhibited comparable figures. These findings came in consistent with previous reports on rabbits (Beynen, 1988; Ayyat, 1991; Fernandez and Fraga, 1992), chickens (Scaife *et al*., 1994), cows (Palmquist and Jenkins, 1980; Whitney *et al*., 2000), fishes (Sener and Yildiz, 2003), and humans (James, 1988), but contradict other reports on chickens (Attia *et al.,* 1994; Soliman *et al.,* 1999; Vieira *et al.,* 2006). It is possible that the disparity between these studies may be attributed to the depressed DM digestibility, insufficiency of metabolizable protein, and low plasma insulin-like growth factor-1 level, as well as to the variation of the dietary vegetable oils, their quality, and the inclusion process or rate. Further studies are probably essential to pinpoint the exact reason. Even though, current results established that feeding rations supplemented with vegetable oils to rabbit bucks didn't elicited any negative influences on their healthiness.

Most importantly, the collected evidences proposed that supplementing vegetable oil-enriched rations to rabbit bucks may demonstrate subsequent positive influences on their reproductive characteristics as early as the 3rd/4th week after feeding on such oils. This conclusion was actually based on the analytical findings of the seminal samples collected once a week from rabbit bucks fed on ration treated with soybean and sunflower oils. According to Castellini and Lattaioli (1999), estimating semen quality is important in determining the reproductive efficiency in rabbit bucks, where it has been documented to be correlated with sperm fertilizing ability (Castellini *et al.,* 2000; Brun *et al.,* 2002; Hagen *et al.,* 2002; Castellini, 2007; Piles *et al.,* 2013; Theau-Clément *et al.,* 2016). The present data indicated that semen collected from rabbit bucks supplemented with vegetable oils had higher sperm concentration, total sperm output, percentage of motile sperms, as well as lower percentages of dead and altered acrosomal sperms than the control bucks. These findings agreed with the ones found in rams (Samadian *et al*., 2010; Esmaeili *et al*., 2012) and boars (Strzezek *et al*., 2004), where it was observed that dietary PUFA effects were prominent between after the 5th weeks of the experiment; thus, suggesting that higher dietary fat levels are important for producing good quality semen in rabbit bucks, from a quantitative and qualitative aspect.

The reason for such observations may relate to two putative mechanisms. The first mechanism at work maybe a direct mechanism; transfers of PUFA from the diet to the testes. Polyunsaturated fatty acids (PUFA) of ω_3 series (like linolenic acid and Docosahexaenoic acid) and ω_6 series (like linoleic acid) are essential for the reproductive activity, where they were found to represent about 30-50% of total amount of fatty acid level in the membrane of animal spermatozoa, which subsequently contribute in enhancing sperms morphology and movement, and thus higher acrosomal responsiveness (Poulos *et al.,* 1973; Wathes *et al.,* 2007). Therefore, PUFA can affect phospholipids composition in the plasma membranes of the testes and altered the availability of gonadotropin receptors, which influenced the rate of testosterone synthesis (Martin *et al*., 1994, 2010; Esmaeili *et al*., 2012). Actually, a tremendous number of articles had showed that a higher degree of PUFA in the spermatozoal membrane augment sperms quantity and concentration in rabbits (Castellini *et al.,* 2003), guinea pigs (Fleming and Yanagimachi, 1984), quails (Al-Daraji *et al.,* 2010), ducks (Ghonim *et al.,* 2010), chickens (Blesbois *et al.,* 1997; Kelso *et al.,* 1997; Cerolini *et al.,* 2000; Bongalhardo *et al.,* 2009), turkey (Zaniboni and Cerolini, 2009), boars (Waterhouse *et al.,* 2006; Hossain *et al.,* 2007; Estienne *et al.,* 2008), horses (Squires, 2006), and humans (Nissen and Kreysel, 1983; Suleiman *et al.,* 1996; Lenzi *et al.,* 2000).

Several articles have also been published, however, indicating that such dietary enrichment of vegetable oils simultaneously increases the susceptibility of spermatozoa to reactive oxygen species and peroxidation, which is one of the major causes of male infertility (Aitken *et al.,* 1993; Wathes *et al.,* 2007). According to Hui (1996), vegetable oils (especially soybean oil, sunflower oil and cottonseed oil) have the highest level of vitamin E (α-Tocopherol) of all the leading oils being used. In fact, they are considered one of the naturally excellent sources of vitamin E (Cheeke and Cunha, 1987; McDonald *et al.,* 2011). Concerning the maintenance of sperm quality during spermatogenesis, epididymal storage and ejaculation, numerous studies have confirmed that there is a strong relationship between the action of vitamin E and the reduction of sperm cells damage. As a matter of fact, Zaniboni and Cerolini (2009) indicated that enrichment of turkey spermatozoa with PUFA and vitamin E by dietary treatment prevents the negative effects of storage on sperm quality and *in vitro* peroxidation. Naseem *et al*. (2007) reported, as well, an improvement of spermatozoa characteristics in rats supplemented with dietary vitamin E and soybean oil. Moreover, daily supplementation with ω_3, ω_6 and ω_9 fatty acids together with vitamin E in male dogs for a period of 60 days considerably increased semen quality and fertilizing ability (Rocha *et al.,* 2009). Thereby, these evidences collectively may attribute the subsequent positive influence of feeding vegetable oils on the spermatogenesis in rabbit bucks to their content of PUFA as well as to their content of vitamin E that could act as an antioxidant through its ability to pass through sperm membranes and then interrupt chain reactions that lead to free radical formation. Further researches are, therefore, definitely imperative to determine the balance between the levels of dietary PUFA consumed and the oxidative stability of semen collected from rabbit bucks.

The second putative mechanism that could explain the obtain results herein could be an indirect effect of PUFA throughout gene expression stimulation of the some enzymes (like elongase and desaturase) that creates a rise in the level of these oil in the sperm. In fact, Esmaeili *et al*. (2012) have confirmed such mechanism in rams. To our knowledge, however, no studies to date have focused on sperm PUFAs persistency or even compared the effect of feeding

refined vegetable oils such as soybean and sunflower oils with PUFA sources on rabbit bucks sperm fatty acids profile. This understanding is essential to link the cascade of events during feeding and after removing the PUFA source from the diet in rabbit bucks. Further researches are definitely warranted.

In conclusion, exploring new feeding strategies are a necessary aspect for improving the reproductive performance in rabbits. Based on the obtained results, feeding rations supplemented with soybean and/or sunflower oils at the level of 3% of DM to rabbit bucks during their adulthood period would produced a good semen quality compared to the control bucks as early as the 3rd/4th week after feeding on such oils. However, further researches are definitely imperative because of the number of bucks per group was considerably low in the current experiment. As a matter of fact, research is currently being undertaken to assert and establish the evaluation of the fertility of buck rabbits fed treated diets on does to confirm the obtained results herein on the reproductive fecundity efficiency of bucks receiving such refined vegetable oils. Research dealing with such aspects may improve our understanding of the nutritional requirements and production of rabbits.

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Conflict of interest

The authors declare no conflicts of interest exist that are of influence on this work.

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