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L-CARNITINE SUPPLEMENTATION FOR AGE-INDUCED REPRODUCTIVE CRITERIA IN MALE PIGEONS

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ABSTRACT

The objective of this study is showing the effect of bird's age on semen quality, semen collection, seminal plasma characteristics and testicular histology on pigeon males, and the potential effect of L-carnitine supplementation on these traits. Forty male pigeons of two ages (1 or 5-years-old) were used in the current study. The pigeons were housed individually and assigned within age, to two L-carnitine treatments (0 or 2 g/L of water) in a 2 x 2 factorial arrangement. There were ten birds per treatment in each age. Collection semen traits, testes mass, sperm parameters, seminal plasma parameters, cloacal mucosa color, donor response to semen collection and testes histology were recorded during the last two weeks of experimental period (10 weeks). The results showed that most of studied traits were depressed with aged birds without L-carnitine supplementation. L-carnitine supplementation to adult and aged birds for 8 weeks significantly increased ejaculate number, collection rate, semen volume, sperm concentration, sperm motility, live sperms, total carnitine in seminal plasma, red cloacal mucosa color percentage, and response to semen collection. Also, L-carnitine supplementation decreased dead sperms, abnormal sperms, seminal plasma total protein, and acid phosphatase activity. Both of L-carnitine supplementation and males' age interacted significantly to influence most of studied traits where the best results were noticed with the adult birds received L-carnitine while the worst records were observed with aged birds without L-carnitine supplementation. Although, absolute and relative testes weights were not affected by either of bird's age or L-carnitine supplementation, the histological examination of testicular tissue of adult or aged birds supplemented with L-carnitine showed a reduction in spermiophage cells and the interstitial cells appeared dark stained indicating high steroid activity. In conclusion, it is recommended that the level of 2g/L L-carnitine could be supplemented to pigeon males to improve semen quality, semen collection, seminal plasma characteristics and testicular histology, especially with aged birds.

Keywords : L-carnitine – pigeon age - semen quality.

INTRODUCTION

The increasing demand of animal protein sources for human consumption can be partially met through pigeon production. Rearing commercial breeds of pigeons for meat is an important branch of small livestock breeding, primarily in the developing countries. Pigeon meat is a healthy food and it is recommended to patients with digestive disorders. Economical production is based on flocks of fatting breeds that mature early, adequate fertility and longer egg production life with respect to usability of parental pairs for more breeding years (Pavieie *et al.,* 2004). If such pigeons are provided with optimal housing conditions, they must be fit for breeding 3 to 4 years. Recently, the avian flu spread and the role of pigeon as a carrier was recognized. For this reason, the housing system could be altered into another style. Since the thought that reared pigeon in closed system leads to decrease reproductive performance and fertility, it is suggested to supply the

pigeon reared in the closed system with an additive that maintain fertility status at the optimal level especially with age progress.

Ageing has different effects on the reproductive system. In the testes, spermatogenesis and steroidogenesis decrease with old age (Levy *et al.,* 1999, Zirkin and Chen, 2000). In addition, in the epididymal epithelium, some striking segment-specific changes occur at the histological and biochemical levels prior to the major loss of spermatogenesis. In male of Japanese quail, Ottinger *et al.,* (2002) reported a loss of fertility, increase of morphological abnormalities in the testes, and a higher incidence of Sertoli cell tumors during aging. Also, the age is considered as an impairment of body function over time caused by accumulation of molecular damage in DNA, proteins and lipids. The accumulating damage may be eventually manifested in age-related health issues, such as decreased fertility (Sampson *et al.,* 2007). It is well known that fertility is a measurable feature that reflects semen quality (Sexton, 1983). Such decreasing fertility problem can be solved by L-carnitine supplementation which may participate to some extent in solving such problem. The merits of semen evaluation in poultry breeding for routinely monitoring their reproductive performance are well recognized (Cheng *et al.,* 2002).

L-carnitine (β-OH-[γ-N-trimethylamino] - butyrate) is a small molecular weight-water soluble amine which occurs naturally in microorganisms, plants and animals (Bremer, 1983). L-carnitine is synthesized from lysine and methionine, the former providing the carbon framework while the latter supplies the methyl group. Supplementation of L-carnitine has been shown to improve semen traits by preventing oxidation of sperm membranes (Neuman *et al.,* 2002). Carnitine has antioxidant properties which may protect sperm membranes from toxic oxygen metabolites. Although the effects of dietary carnitine on carcass composition (Barker and Sell, 1994), egg production, and hatchability (Leibetseder, 1995) have been assessed in poultry, the effect of this supplements on the reproductive performance of the avian breeder males are minute and need more investigation. Thus, the objective of this study is showing the effect of bird's age on semen quality, semen collection, seminal plasma characteristics and testicular histology on pigeon males, and the potential effect of L-carnitine supplementation on these traits.

MATERIALS AND METHODS

Management and housing:

Local pigeons which had nearly the similar weights were obtained from private farms, wing banded, individually weighed and housed individually in separate metal cages (60 x 50 x 35 cm) in open-sided shelter. Birds received 16 hr light daily throughout the experimental period. A conventional corn-bean basal diet [18.8% crude protein and 3300 kcal/g metabolizable energy (Janssens., *et al.,* 1998)] was used. Feed and water were available *ad libitum*. All birds were kept under the same management, hygienic and environmental conditions throughout the entire experimental period that lasted for 10 weeks.

Experimental procedure:

Forty male pigeons of two ages (1 or 5-years-old) were used in the current study. The pigeons were housed individually and assigned within age, to two L-carnitine treatments (0 or 2 g/L of water) in a 2 x 2 factorial arrangement. There were ten birds per treatment in each age. L-carnitine was obtained from private pharmaceuticals company in Cairo.

Semen traits:

Semen collection and seminal plasma:

Semen was collected by the conventional massage method (Lake and Stewart, 1978). Attempts were made to collect semen from each male twice weekly during the last two weeks of experimental period. Attempt and ejaculate numbers were recorded for each treatment. After collection, it was immediately centrifuged at 15000 rpm for 3 min., and the resultant supernatant was used as the seminal plasma. The protein concentration of seminal plasma was measured by the Lowry method (Lowry *et al.,* 1951). Acid phosphatase activities in seminal plasma were measured using p-nitrophenyl phosphate as a substrate (Bessey *et al.,* 1946). Total carnitine was determined according to the method described by Deufel, (1990).

Semen quality evaluation:

Volume of semen was measured when aspirated from the cloacal vent by using a micropipette. Sperm concentration was calculated with a hemocytometer after dilution of the semen (1:200) with a weak eosin solution (Brillard and McDaniel, 1985). The sperm motility was estimated by microscopic observation. Motility was expressed as the percentage of motile spermatozoa with moderate to rapid progressive movement (Sexton, 1977). Sperm morphology was examined microscopically in smears stained with nigrosin and eosin (Blom, 1950). The proportions of live (eosin-impermeable) and dead (eosin-permeable) spermatozoa in sample were assessed on the basis of 200 spermatozoa.

Submission and cloacal mucosa color evaluation:

According to the method described by Cheng *et al.,* (2002), Semen collection sessions were subjectively classified as submissive, tolerant, or resistant, for donor response. Pigeons usually respond to massage by waving the tail and showing the cloacal opening. If the pigeon was calm and submissive to operator's handling, it was subjectively classified as submissive. Those that responded slightly by struggling but allowed collection were classified as tolerant. If the bird vigorously responded to the handling by struggling or flapping and attempted to escape, it was classified as resistant. The everted membrane folds exhibited various degrees of redness, subjectively classified as red, pink, or pale.

Histological study:

At the end of the experimental period, 5 birds per each treatment were weighed and slaughtered. Testes were excised and weighed. Testes weights were expressed relative to body weight. The left testis of each bird was cut into serial cross sections 5 mm in thickness and fixed in 10% neutral buffered formalin. Fixed samples were processed and stained with hematoxylin and eosin (Prophet *et al.,* 1994). Three preparations of each left testis of each bird were examined microscopically.

Statistical analysis:

The general linear models procedure of the SAS system was utilized (SAS Institute, 1988). Significant differences between means were determined by Dancan's multiple-range tests (Duncan, 1955).

RESULTS

Effect of age on collection semen traits:

Semen collection characteristics data of pigeon males as influenced by age are illustrated in Table (1). The effect of age *per se* was recognized where aged birds (5-years-old) showed significant decrease in ejaculate number (22.1%), collection rate (22.1%) and semen volume (27.5%) compared with the adult birds (1-year-old).

Table (1): Effect of bird's age and L-carnitine supplementation on collection semen traits:

Traits / Treatments			Collection attempts (n)	Ejaculate (n)	Collection rate (%)	Semen Volume (µL)
Age	Adult		80	48 [a]	60.00 [a]	13.08 [a]
	Aged		80	37 [b]	46.25 [b]	9.46 [b]
	SE			0.67	1.42	0.04
L-Carnitine (g/l)	0		80	38 [b]	47.50 [b]	10.25 [b]
	2		80	47 [a]	58.75 [a]	12.28 [a]
	SE			0.68	1.69	0.04
Interaction	Adult	0	40	23 [a]	57.50 [a]	12.59 [a]
		2	40	25 [a]	62.50 [a]	13.56 [a]
	Aged	0	40	15 [b]	37.50 [b]	7.91 [b]
		2	40	22 [a]	55.00 [a]	11.00 [a]
	SE			0.96	2.39	0.06
Significant	Age			**	**	**
	L-Carnitine			**	**	*
	Age x L-Carnitine			*	*	*

Means within column for each item having different superscript differ significantly
*** (p≤0.05) ** (p≤0.01)**

Effect of L-carnitine on collection semen traits:

The effect of L-carnitine administration *per se* on semen collection characteristics are tabulated in Table (1). Birds supplemented with L-carnitine were significantly increased ejaculate number by 24.6% compared to the unsupplemented birds. Similar trend was noticed also with collection rate (24.6%) and semen volume (18.5%).

Interaction:

The interaction of age x L-carnitine was analyzed. Age x L-carnitine interacted significantly to affect the all semen collection traits. The higher values were noticed with adult birds received 2 mg/L L-carnitine while the lower values were observed with aged birds without L-carnitine supplementation.

Effect of age on testes mass:

Results of testes mass of birds at two different ages summarized in Table (2). Statistical analysis revealed no differences in absolute and relative testes weights.

Table (2): Effect of bird's age and L-carnitine supplementation on testes weight:

Treatments \ Traits		Live body weight (g)	Absolute testes weight (g)	Relative testes weight (%)
Age	Adult	302.55	11.70	3.87
	Aged	309.80	12.12	3.91
	SE	0.66	0.11	0.14
L-Carnitine (g/l)	0	305.90	11.59	3.79
	2	306.45	12.23	3.99
	SE	0.62	0.21	0.18
Interaction	Adult 0	301.90	11.16	3.69
	Adult 2	303.20	12.24	4.04
	Aged 0	309.90	12.02	3.88
	Aged 2	309.70	12.21	3.94
	SE	0.94	0.26	0.24
Significant	Age	N.S	N.S	N.S
	L-Carnitine	N.S	N.S	N.S
	Age x L-Carnitine	N.S	N.S	N.S

Effect of L-carnitine on testes mass:

Concerning testes mass, it can be noticed from Table (2) that testes mass were not influenced by L-carnitine supplementation.

Interaction:

Testes weight did not influenced by the interaction of L-carnitine supplementation and males' age.

Effect of age on sperm parameters:

Data of sperm parameters are presented in Table (3). Sperm concentration, sperm motility and live sperms were significantly decreased in aged males by 20%, 20.2% and 5.9%, respectively, compared to adult males. Dead and abnormal sperms were significantly increased in aged males by 40.2% and 30.2%, respectively, compared to adult males.

Effect of L-carnitine on sperm parameters:

The data of sperm parameters of pigeon males as affected by L-carnitine supplementation are summarized in Table (3). L-carnitine supplementation led to increase sperm concentration, sperm motility and live sperms by 18.2%, 10% and 4.8%, respectively, and decrease dead and abnormal sperms by 18.3% and 25.7%, respectively, compared with unsupplementation birds.

Interaction:

The interaction of age x L-carnitine were studied. The best results of sperm concentration, sperm motility, live sperms, dead sperms and abnormal sperms were noticed with adult birds received L-carnitine while the worst results were observed in aged birds without L-carnitine.

Table (3): Effect of bird's age and L-carnitine supplementation on sperm parameters:

Traits / Treatments		Sperm concent. (10^9)	Sperm motility (%)	Live sperms (%)	Dead sperms (%)	Abnormal sperms (%)
Age	Adult	3.95 a	79.74 a	85.99 a	8.68 b	5.33 b
	Aged	3.20 b	63.61 b	80.93 b	12.22 a	6.85 a
	SE	0.03	0.22	0.17	0.09	0.08
L-Carnitine (g/l)	0	3.30 b	68.29 b	81.53 b	11.46 a	7.01 a
	2	3.86 a	75.07 a	85.40 a	9.44 b	5.17 b
	SE	0.19	0.22	0.25	0.10	0.09
Interaction	Adult 0	3.69 a	78.47 a	83.76b	9.81 b	6.43 a
	Adult 2	4.21 a	81.01 a	88.23 a	7.55 b	4.22 b
	Aged 0	2.90 b	58.10 c	79.30 b	13.11 a	7.59 a
	Aged 2	3.50 a	69.12 b	82.55 b	11.34 a	6.11 b
	SE	0.04	0.31	0.34	0.14	0.12
Significant	Age	*	**	*	**	*
	L-Carnitine	*	*	*	*	*
	AgexL-Carnitine	*	*	*	*	*

Means within column for each item having different superscript differ significantly
*** (p≤0.05) ** (p≤0.01), Sperm concent. = Sperm concentration per ejaculate.**

Effect of age on seminal plasma parameters:

The results of the determined seminal plasma parameters are presented in Table (4). The higher seminal plasma total protein and acid phosphatase activity were noticed with aged birds, it increased by 30.2% and 62.9%, respectively, compared to adult birds. Total carnitine in seminal plasma not affected by age.

Table (4): Effect of bird's age and L-carnitine supplementation on biochemical seminal Plasma:

Traits / Treatments		Total protein Concentration (mg/ml)	Acid phosphatase Activity (U/L)	Total carnitine (µmoles/l)
Age	Adult	17.92 b	2216.05 b	13.49
	Aged	23.34 a	3610.70 a	13.55
	SE	0.13	1.71	0.08
L-Carnitine (g/l)	0	23.40 a	3127.80 a	11.09 b
	2	17.87 b	2698.95 b	15.95 a
	SE	0.15	1.56	0.08
Interaction	Adult 0	20.63 b	2311.60 c	11.28 b
	Adult 2	15.21 c	2120.50 c	15.69 a
	Aged 0	26.16 a	3944.00 a	10.89 b
	Aged 2	20.52 b	3277.40 b	16.20 a
	SE	0.19	2.42	0.11
Significant	Age	**	**	N.S
	L-Carnitine	**	*	*
	Age x L-Carnitine	*	*	*

Means within column for each item having different superscript differ significantly
*** (p≤0.05) ** (p≤0.01)**

Effect of L-carnitine on seminal plasma parameters:

Seminal plasma data of pigeon males as influenced by L-carnitine supplementation are illustrated in Table (4). L-carnitine supplementation significantly decreased seminal plasma total protein and acid phosphatase activity by 23.6% and 13.7%, respectively, while total L-carnitine was increased by 65.4% compared to unsupplemented birds.

Interaction:

Age and L-carnitine interacted significantly to influence total protein, acid phosphatase and total L-canitine in seminal plasma. The higher values were noticed for aged birds without L-carnitine, while the lower values were observed in adult birds received L-carnitine.

Effect of age on cloacal mucosa color:

Results of cloacal mucosa color of pigeon males as affected by age are summarized in Table (5). Adult birds recorded the highest percentage of red cloacal mucosa color and the lowest percentage of pale cloacal mucosa color compared with the aged birds. All differences were significantly.

Table (5): Effect of bird's age and L-carnitine supplementation on cloacal mucosa color:

Traits / Treatments		Red (%)	Pink (%)	Pale (%)
Age	Adult	46.25 ^{a}	45.00 ^{a}	7.50 ^{b}
	Aged	40.00 ^{b}	42.50 ^{b}	20.00 ^{a}
	SE	0.47	0.23	0.15
L-Carnitine (g/l)	0	52.50 ^{a}	40.00 ^{b}	8.75 ^{b}
	2	33.75 ^{b}	47.50 ^{a}	18.75 ^{a}
	SE	0.33	0.25	0.19
Interaction	Adult 0	55.00 ^{a}	40.00 ^{b}	5.00 ^{c}
	Adult 2	37.50 ^{b}	50.00 ^{a}	10.00 ^{b}
	Aged 0	50.00 ^{a}	40.00 ^{b}	12.50 ^{b}
	Aged 2	30.00 ^{c}	45.00 ^{a}	27.50 ^{a}
	SE	0.51	0.40	0.27
Significant	Age	*	*	**
	L-Carnitine	**	**	**
	Age x L-Carnitine	*	*	*

Means within column for each item having different superscript differ significantly
*** (p≤0.05) ** (p≤0.01)**

Effect of L-carnitine on cloacal mucosa color:

Concerning cloacal mucosa color, it can be observed from Table (5) that cloacal mucosa color was influenced significantly by L-carnitine supplementation, where L-carnitine supplementation increased red color by 52.2% and decreased pink and pale colors by 16.2% and 54.9%, respectively, compared with unsupplemeted birds.

Interaction:

L-carnitine supplementation and bird's age interacted significantly to influence cloacal mucosa color. The best results were noticed with the adult

birds received L-carnitine while the worst records were observed with aged birds without L-carnitine supplementation.

Effects of age on donor response to semen collection:

The effect of bird's age on donor response to semen collection is presented in table (6). The results showed that submissive percentage was lower by 15.2% while the resistant percentage was higher by 25.9% in aged birds compared with the younger ones.

Table (6): Effect of bird's age and L-carnitine supplementation on donor response for semen collection:

Treatments \ Traits		Submissive (%)	Tolerant (%)	Resistant (%)
Age	Adult	51.91 ^{a}	33.39	14.69 ^{b}
	Aged	45.00 ^{b}	38.18	16.82 ^{a}
	SE	0.18	0.42	0.09
L-Carnitine (g/l)	0	43.91 ^{b}	37.39	18.70 ^{a}
	2	53.30 ^{a}	34.18	12.82 ^{b}
	SE	0.11	0.51	0.07
Interaction	Adult 0	47.82 ^{b}	34.78	17.39 ^{a}
	Adult 2	56.00 ^{a}	32.00	12.00 ^{b}
	Aged 0	40.00 ^{b}	40.00	20.00 ^{a}
	Aged 2	50.00 ^{b}	36.36	13.64 ^{b}
	SE	0.35	1.21	0.91
Significant	Age	*	N.S	*
	L-Carnitine	*	N.S	*
	Age x L-Carnitine	*	N.S	*

Means within column for each item having different superscript differ significantly
*** (p≤0.05)**

Effect of L-carnitine on donor response to semen collection:

Donor response to semen collection data of pigeon males affected by L-carnitine supplementation are summarized in Table (6). The effect of L-carnitine supplementation was recorded where birds received L-carnitine showed significant increased in submissive percentage by 18.7% and significant decreased in resistant percentage by 40.3% compared with birds without L-carnitine supplementation. Tolerant percentage did not significantly influenced by L-carnitine supplementation.

Interaction:

The interaction of L-carnitine x bird's age was analyzed. L-carnitine x birds' age interacted significantly to affect the donor response to semen collection. The best results were observed in adult birds received L-carnitine (the highest submissive percentage and the lowest resistant percentage), while the worst results were observed in aged birds without L-carnitine supplementation. Tolerant percentage did not influenced by the interaction of L-carnitine supplementation and bird's age.

Histological study results:

Differences among treatments were noted in the histological examination of testicular tissue (Figure 1). L-carnitine supplementation with

adult or aged birds showed a reduction in spermiophage cells and advanced degree of spermatogenesis with formation of numerous sperm cells in the center of the tubules. Also, interstitial cells appear in the triangle between three adjacent tubules, the cells appear dark stained indicating high steroid activity. The same results were noted in adult birds without L-carnitine supplementation. But histological examination of testicular tissue of aged birds showed spermiophage cell and the Interstitial cells appear pale stained indicating low steroid activity.

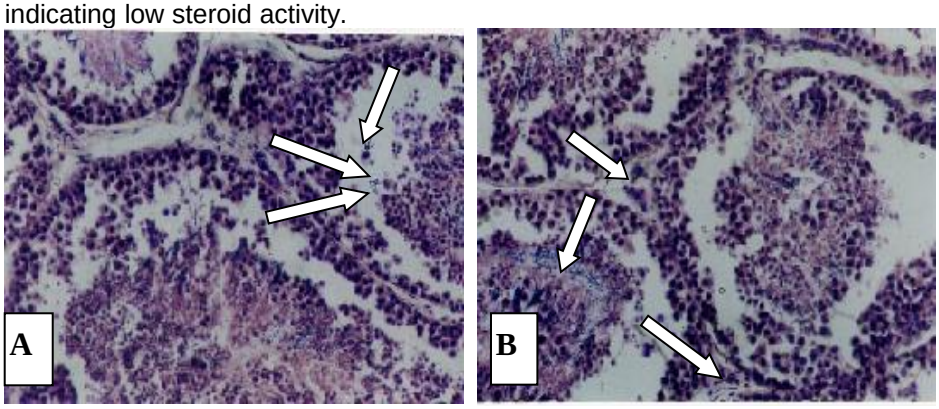

Figure (1): left testes of pigeon males A)- aged pigeon male (without L-carnitine supplementation) testis. Magnification is 400x. Arrows indicate spermiophage cells. B)- Testis from a pigeon males (adult or aged) supplemented with L-carnitine. Magnification is 400x. Arrows indicate dark interstitial cells and numerous sperm cells. Note the absence of spermiophage cells.

DISCUSSION

As previously mentioned, the aim of the present study was to show the effect of bird's age on semen quality, seminal plasma characteristics and testicular histology on pigeon males, and the potential effect of L-carnitine supplementation on these traits. The current results showed that collection semen parameters were decreased in response to age progress. These results are in line with those found by Sampson *et al.,* (2007) who pointed out that the progressing of age produced an altered testicular function and changes in semen output and fecundity. These changes may be related to altered steroidogenesis of the aging testes, ultrastructural histomorphological changes at the testicular level and functional and morphological compromises of the aging spermatozoa. In the same direction, Kelso *et al.,* (1996) found that poultry species quality parameters changed with age leading to a progressive decline in fertility. Lukaszewicz *et al.,* (2004) stated that ejaculate volume decreased with the age while sperm concentration increased. Retterstol *et al.,* (2001) demonstrated that aging was accompanied by a decrease in docosahexaenoic acid proportion in the sperm phospholipids where DHA is considered as a major component of the sperm cell membranes.

The observed data showed that ejaculate number, collection rate and semen volume improved significantly with L-carnitine supplementation. These results are in line to some extent with the results obtained by Baumgartner, (1998) who reported that L-carnitine supplementation increased the semen volume vs. control. The improvement of the collection semen traits may be due to the medical properties of L-carnitine where L-carnitine acted as antioxidant. The antiperoxidant activity is dependent on the presence of antiperoxidant factors which may attach to the spermatozoa at ejaculation and can protect them from lipid peroxidative attacks during their passage through the reproductive system (Strzezek *et al.,* 2000). Because L-carnitine acts as antioxidant, it thought that L-carnitine may prevent hypothalamus cells from the degradation as a result of oxidative process during the age progress. Consequently, L-carnitine indirectly affected semen collection.

Absolute and relative testes weights were not influenced by L-carnitine supplementation, birds' age and the interaction of L-carnitine supplementation with males' age. These results are in harmony with those reported in roosters by Neuman *et al.,* (2002). Who found that roosters testes weight was unaffected by L-carnitine supplementation.

The decrease of sperm parameters in the current study was anticipated in aged birds. It could be concluded that sperm concentration, sperm motility and live sperms were significantly decreased in aged males. The dead and abnormal sperms were significantly increased in aged males. The current results supported the results obtained by Keslo *et al.,* (1996) who pointed out that ejaculate volume and sperm concentration are dependent on age of males. Sperm mobility phenotype was independent of age in New Hampshire roosters (Froman *et al.,* 1997; Froman and Feltmann, 1998).

L-carnitine supplementation improved most sperm parameters in the current investigation. Theses results are compatible with the results observed by Xin-Zhou *et al.,* (2007) who pointed out that L-carnitine showed some considerable positive effects in improving sperm quality. The current results support the results obtained by Neuman *et al.,* (2002) who observed that L-carnitine supplementation induced higher sperm concentration and lower dead sperm percentage than control birds. They hypothesized that roosters supplemented L-carnitine showed an improvement in semen traits by preventing oxidation of sperm membranes. The results of Aitken *et al.,* (1993) confirmed that sperm plasma membrane is susceptible to lipid peroxidation. Recent evidence has shown that oxidative stress significantly impairs sperm function and plays a pivotal role in the etiology of defective sperm function (Jerzy Strzezek *et al.,* 2004). L-carnitine, a vitamin like antioxidant, plays a pivotal role in the maturation of spermatozoa within the reproductive tract (Dikmen *et al.,* 2006). Some evidence suggests a key role of L-carnitine for sperm motility. L carnitine may be also responsible for removing excess intracellular toxic acetyl-CoA, which protects spermatozoa from oxidative damage (Xin-Zhou *et al.,* 2007). A possible explanation for the increase in sperm concentration of L-carnitine supplementation birds was that L-carnitine facilitated the preservation of the sperm lipid membranes, thereby extending sperm longevity.

The higher seminal plasma total protein and acid phosphatase activity were noticed with aged birds compared to adult birds. L-carnitine supplementation significantly decreased seminal plasma total protein and acid phosphatase activity compared to unsupplemented birds. Age and L-carnitine interacted significantly to influence total protein, acid phosphatase and total canitine in seminal plasma. The higher values were noticed for aged birds without L-carnitine, while the lower values were observed in adult birds received L-carnitine. Kotlowska *et al.,* (2005) reported that biochemical parameters of semen are more sensitive markers of semen quality than quantitative semen parameters. Because the increased changes in biochemical parameters may originate from damaged spermatozoa, it seems reasonable to hypothesize that biochemical semen parameters are rather linked with sperm morphology and live status characteristics. The elevated ACP activity of seminal plasma at the end of reproductive period may be related to changes occurring in the turkey reproductive tract at the end of reproductive period or can originate from damaged spermatozoa. The ACP activity in turkey spermatozoa was positively correlated with activity in seminal plasma, total number of sperm, and ejaculate volume. Our results are in line with those found by Zopfgen *et al.,* (2000) who observed clearly reduced concentration of total L-carnitine in infertile men compared with control.

The current study showed that adult birds possessed the highest percentage of red cloacal mucosa color and the lowest percentage of pale cloacal mucosa color compared with the aged birds. The results of Lee *et al.,*(1999) showed that ejaculation rarely occurred when the color of cloacal mucosal membranes was pale, and less semen was ejaculated. They also added that sexual arousal was not sufficiently with a pale color of cloacal mucosal membrane. Knight *et al.,* (1984) observed, during semen collection, that the edematous phallic folds are visible and the mucosal folds are engorged with lymphatic fluid. The filling of the sinus network under the phallic folds resulted in varying degrees of redness of the cloacal mucosa surface, indicating the level of sexual arousal. From the current results, it can be noticed also that cloacal mucosa color was influenced significantly by L-carnitine supplementation, where L-carnitine supplementation increased red color and decreased pink and pale colors compared with unsupplemeted birds. Redness degree of the cloacal mucosa is considered as an indication of sexual arousal that leads to ejaculation. Consequently, L-carnitine may affect the status of sexual arousal that followed by increased in redness degree of the cloacal color.

L-carnitine-supplementation birds had significantly fewer spermiophage cells per testes than aged birds without L-carnitine supplementation. These results are in line with those found by Neuman *et al,.* (2002) who found that L-Carnitine-fed birds had significantly fewer multinucleated giant cells (MGC) per testes than control-fed birds. Testicular MGC are described as a degenerative syndrome resulting presumably from the inability of tetraploid primary spermatocytes to complete meiotic division; thus, maturation arrests at the spermatid stage of development (Bloom and Fawcett, 1975; Corrier *et al.,* 1985). In the current study, L-carnitine supplementation males showed

advanced degree of spermatogenesis with formation of numerous sperm cells in the center of tubules. Corrier *et al.,* (1985); Sur *et al.,*(1997) reported that Multinucleated giant cells are composed primarily of aggregates of degenerated spermatocytes and spermatids and are often sloughed into the lumen of seminiferous tubules.

CONCLUSION

It could be concluded that L-carnitine supplementation improved most of collection semen traits, sperm parameters, seminal plasma parameters, cloacal mucosa color, donor response to semen collection and testes histology in pigeon males, especially with aged males.

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تهدف هذه الدراسة الى بيان التأثيرات السلبية للتقدم فى العمر على صفات السائل المنوى ومكونات بلازما السائل المنوى والتركيب الهستولوجى للخصية فى ذكور الحمام، ومدى امكانية تحسين هذه الصفات عن طريق امداد الطيور بمادة الكارنيتين فى مياه الشرب. استخدم فى هذه الدراسة 40 من ذكور الحمام من عمرين مختلفين(20 منهم عمر عام واحد & 20 منهم عمر 5 سنوات). تم اسكان الطيور فى أقفاص فردية، حيث تم تقسيم الطيور داخل كل عمر الى مجموعتين (كل مجموعة 10 ذكور) قدم لهما صفر & 2 جرام / لتر ماء من مادة الكارنيتين. تم التسجيل لصفات جمع السائل المنوى، ووزن الخصية، وصفات السائل المنوى، وصفات بلازما السائل المنوى، ولون المجمع، ونسبة الاستجابة لعملية تجميع السائل المنوى، كما أجريت دراسة هستولوجية على الخصية. أظهرت النتائج انخفاض معظم الصفات تحت الدراسة فى حالة الطيور المتقدمة فى العمر (5 سنوات) والتى لم يقدم لها مادة الكارنيتين. أدت اضافة مادة الكارنيتين فى مياه الشرب للطيور الناضجة أو المتقدمة فى العمر لمدة 8 أسابيع الى زيادة معدل تجميع السائل المنوى، وحجم السائل المنوى، وتركيز وحيوية الاسبرمات، ونسبة الاسبرمات الحية، ونسبة الكارنيتين فى بلازما السائل المنوى، ونسبة اللون الأحمر للمجمع، والاستجابة لعملية التجميع. كما أدى استخدام الكارنيتين الى انخفاض نسبة الاسبرمات الميتة والمشوهة، و كذلك البروتين الكلى فى بلازما السائل المنوى ونشاط الأسد فوسفاتيز. أظهرت النتائج أيضا وجود تداخل معنوى بين عمر الطيور واضافة الكارنيتين وأثر ذلك معنويا على أغلب الصفات تحت الدراسة حيث أظهرت الطيور الناضجة حديثا والتى قدم لها مادة الكارنيتين أفضل النتائج، فى حين لوحظت أسوء النتائج مع الطيور المتقدمة فى العمر والتى لم يقدم لها الكارنيتين. وبالرغم من أن كل من الوزن المطلق والنسبى للخصية لم يتأثر بعمر الطيور أو باضافة الكارنيتين، الا أن الفحص الهستولوجي لخصية الطيور الناضجة أو المتقدمة فى العمر والتى تناولت الكارنيتين أوضحت انخفاض فى عدد الخلايا الأكولة، كما ظهرت الخلايا البينية بصورة داكنة مما يدل على ارتفاع النشاط الاستيرودى لها. مما سبق يتضح أن اضافة 2 جرام من مادة الكارنيتين /لتر من مياه الشرب أدى الى تحسين صفات جمع السائل المنوى، صفات السائل المنوى ، بلازما السائل المنوى، الصفات الهستولوجية لخصية ذكور الحمام، وبخاصة فى حالة الطيور المتقدمة فى العمر.