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EFFECT OF DIETARY SUPPLEMENTATION WITH LETTUCE AND CABBAGE BY- PRODUCTS ON FRESH AND COOLED RABBITS SEMEN.

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ABSTRACT: *Using agricultural by-products in livestock feeds can help farmers reduce feed costs and help food waste generators reduce disposal costs while minimizing the environmental problems associated with this waste.* Forty-two New *Zealand White rabbit bucks aged 8 months were randomly divided into 7 groups. First group served as control group was fed on the basal diet. Second, 3rd and 4th groups were fed a basal diet supplemented with 1, 2 and 3 % dried lettuce (LT) by-product. The 5th, 6th and 7th groups were fed the basal diet supplemented with 1, 2 and 3% dried cabbage (CB) by-product. Two experiments were performed. The 1st experiment investigated the e*ffect *of dietary* supplementation *with LT and CB on fresh semen quality parameters, including volume, motility %, viability, abnormalities, acrosomal damage, and sperm concentration. The 2nd experiment examined the effect of LT and CB supplementation as well as the storage time on the previous semen quality parameters of cooled semen during storage at 5° C up to 72 hours. Enzymes activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured.*

In 1st expriment, results showed that sperm concentration increased (P<0.01) in all LT or CB supplemented groups. However, % of dead and abnormal spermatozoa decreased (P<0.05). Ejaculate volume and motility % increased (P<0.05) in 3% LT supplemented group. Experiment 2 showed that motility percentages increased (P<0.01), however, percentages of dead spermatozoa decreased (P<0.01) in bucks supplemented with 1% and 3% LT compared to control. Also, CB supplementation increased (P<0.01) the percentage of motility while decreased (P<0.01) the percentages of dead and acrosomal damage sperm compared to control. Results showed that ALT and AST levels were lower (P<0.01) in all

groups supplemented with LT or CB compared to control. ***Conclusively,*** *the present results demonstrate that, the highest reproductive performance was achieved when bucks fed diet supplemented with 3% of LT and CB by-products.*

Key words*: Semen, rabbits, lettuce, cabbage, by-products.*

INTRODUCTION

In Egypt, animal producers suffer from a shortage of feed resources and raw materials as well as the continuous increases in their prices during the recent years. However, there are great amounts of agricultural by-products that can be used to close the gap of animal feed shortage (Alwan, 2019). Addationaly, using agricultural by-products in livestock feeds can help farmers reduce feed costs (de Evan *et al.,* 2019). Moreover, rabbit digestive system is suitable for the diets that contain low grains and high fiber (Abdel-Aziz *et al.,* 2015). Furthermore, simple biological characteristics, high fecundity and prolificacy, short generation interval and better feed conversion efficiency place rabbit close to poultry in term of feed efficiency (Hasanat *et al.,* 2006). Lettuce- and cabbage by-product can be used successfully as a suitable feedstuff for rabbits (Bakshi *et al.,* 2016; Alwan, 2019). Lettuce (*Lactuca sativa*) belongs to Asteraceae family, tribe Cichorieae, it has many medicinal values (Mou, 2011). Lettuce has some benefits mainly due to the presence of natural antioxidant compounds (vitamins A, C and E, carotenoids, polyphenols) alongside significant amounts of fiber and certain minerals (Garg *et al.,* 2004; Gan and Azrina, 2016; Al-Shmgani *et al.,* 2017). Furthermore, lettuce has sedative, analgesic, hypoglycemic, antifungal as well as antioxidant properties (Garg *et al.,* 2004; Gan and Azrina, 2016; Al-Shmgani *et al.,* 2017). Additionally, cabbage belongs to family Brassicaceae or Cruciferae which includes many eaten vegetables such as cauliflower, broccoli, Brussels sprout, cabbage, and others (Latte *et al.,* 2011; Adeoye *et al.,* 2019a). Cabbage contains many vitamins (especially A, B, C and E) and minerals such as sulfur, calcium, silica, magnesium, iron, iodine, and phosphorus compounds (Bakshi *et al.,* 2016; Adeoye *et al.,* 2019a). It was documented *in vivo* and *in vitro* that cabbage may have some anti cancer and antioxidants properties (Michael *et al.,* 1999).

Artificial insemination (AI) offers many benefits to the livestock industry principally in conjunction with genetic selection programs (Peris *et al.,* 2004). In rabbits, fresh diluted semen can be used, yielding pregnancy rates similar to or less than those obtained in natural mating (Morrell, 1995). However, the fertilizing ability of rabbit sperm used in AI can be reduced due to some detrimental factors produced during semen preservation. Alvarino (2000)

mention that rabbit semen preservation is one of the most common problems for the wide use of AI in industrial Rabbitries.

Semen quality tests are usually used to determine the potential usability of processed semen for breeding programs (Peris *et al.,* 2004). Thus accurate evaluation of these sperm quality tests is of major importance (Peris *et al.,* 2004 & 2007).

To our knowledge, there is no study available, to date, on the effect of lettuce and cabbage by-products on sperm quality parameters of rabbit bucks.

Therefore, the objective of this work was to investigate the effect of addition of different levels of lettuce and cabbage by-products to the diet on semen quality parameters of rabbit bucks. Also, semen quality parameters of cooled semen were recorded during the storage at 5^{O} C throughout 72 hours.

MATERIALS AND METHODS

Animals

A total number of 42 New Zealand White (NZW) bucks rabbits aged 8 months and the body weight mean 3.45 ± 0.25 kg were used in this trial at Rabbitry Farm of Animal Production Departments, Faculty of Agriculture, Zagazig University, Zagazig, Egypt during winter season. Bucks were individually raised in a wire galvanized battery cages (50 × 55 × 40 cm), which supplied with feeders and automatic nipple drinkers. Bucks were reared under the same managerial and hygienic conditions. Light intensity measured at the middle of the cages ranged between 25-30 lux. using incandescent bulbs in semi-closed house. The bucks were randomly divided into seven similar groups (6 bucks/group), all groups were fed *ad libitum* on a commercial pelleted diet (basal diet). The first group was fed on the basal diet without any supplementation and served as a control. The 2^{nd}, 3^{rd} and 4^{th} groups were fed on basal diet supplemented with 1, 2 and 3 % of dried lettuce (LT) by-product. The 5^{th}, 6^{th} and 7^{th} groups were fed on the basal diet supplemented with 1, 2 and 3% dried cabbage by-product (CB) (Mohammadagheri *et al.,* 2016; Feher *et al.*, 2021).

The composition and chemical analysis of the basal diet are presented in Table 1. The chemical composition of dried lettuce and cabbage by-product was determined according to AOAC (2000). Lettuce by-product contained dry matter 86.79 %, crude protein 18.50 %, crude fiber 22.12%, ether extract 1.42 % and ash 20.25 %. Cabbage by-product contained dry matter 88.40 %, crude protein 16.95 %, crude fiber 21.40%, ether extract 2.79 % and ash 18.4 %. All

Table 1. Composition and chemical analysis of the basal diet

Items	
Ingredients: (g/kg)	
Alfalfa hay	18.0
Soybean meal	19.0
Wheat bran	24.5
Barley	22.50
Yellow corn	9.50
Molasses	3.00
Limestone	1.50
Sodium chloride salt	1.70
Vitamin and mineral premix[a]	.30
Total	**100**
Chemical analysis (% as fed basis)	
Crude protein[b]	17.25
Crude fiber[b]	12.23
Neutral detergent fiber[c]	34.61
Acid detergent fiber[c]	16.96
Digestible energy[d]	2651 kcal/kg

[a] **Each kilogram contains the following:** Vit. A 12000 IU, Vit. D3 2200 IU, Vit Vit Vit. E 10.0 mg, Vit. K 2.0 mg, Vit. B1 4.0 mg, Vit B2 1.5 mg, Vit. B5 6.3 mg, Vit.6 1.7 mg, Vit t. B12 0.03 mg, Biotin 3.3 mg, Folic acid 0.83 mg, Cholin 200 mg, Zn 11.79 mg, Mn 5.00 mg, Fe 12.5 mg, I 0.33 mg, Se 0.65 mg, and Mg 66.79 mg

[b] Analyzed according to AOAC (2000)

[c] Analyzed according to Van Soest *et al.* (1991)

[d] Calculated according to NRC (1984)

the experimental protocols were approved by the Research Ethics Committee at the Faculty of Agriculture, Zagazig University, Zagazig, Egypt.

Semen collection and processing:

After five weeks of feeding on the experimental diets, all bucks were trained to ejaculate into artificial vagina (AV) throughout 2 weeks. Semen samples were collected between 7.00 am and 9.00 am. Semen was collected from all bucks artificially once a week for up to 5 weeks by using AV maintained at 40-42^{O}C according to El-Seadawy *et al.* (2017). Following semen collection, each ejaculate from each buck was evaluated individually by using light microscopy. Samples with total sperm motility below 70% were discarded. Semen samples were divided into two parts. The 1st part was diluted

with saline solution (0.9% NaCl) and used as fresh sample for experiment 1. The 2nd part was used for experiment 2.

Ejaculates within each group were pooled to increase semen volume and to avoid individual differences (6 ejaculates/pool; 5 pools/group; 7 groups; 35 pools in total) (El-Kelawy *et al*., 2012). Pooled semen was extended with lactose-yolk-citrate (LYC) extender. LYC is composed of 1.25 g lactose, 2.90 g sodium citrate dihydrate, 0.04 g citric acid anhydrous and 10 ml egg yolk containing 500 IU/ml penicillin G- Sodium and 500 µg/ml streptomycin sulfate per 100 ml distilled water as described by Zeidan *et al.* (2008). The dilution rate was 1 semen: 5 diluent v/v. Diluted semen was then cooled to 5°C over 2 hours and stored at this temperature for 72 hours. Sperm quality parameters were observed during 0, 24, 48, and 72 hours of storage at 5°C in LYC extender.

Experiments:

Two experiments were performed in this study. 1st experiment investigated the effect of dietary supplementation with different levels of LT and CB by-products on fresh semen quality parameters. Semen quality parameters including, motility %, viability, abnormalities, acrosomal damage, and sperm-cell concentration (10^6/ml). Semen ejaculates volume, (ml) and hydrogen-ion concentration (pH) were also evaluated. Sperm motility (%) was evaluated as described previously (Abdel-Wareth *et al*., 2019). Dead sperm () was evaluated by using the eosin/nigrosin staining procedure. Purple-stained sperm were considered dead, whereas non-stained sperm were considered viable (Peris *et al*., 2007; Abdel-Wareth *et al*., 2019). Sperm abnormalities (%) were determined in the same smears prepared to assess dead sperm. The percentage of abnormal sperm was estimated by counting 200 sperm in different microscope fields by using high power magnification (400x) (Abdel-Wareth *et al*., 2019). The percentage of acrosomal damage was evaluated using a light microscope (100x) (El-Sherbieny *et al*., 2012). Semen-ejaculate volume (ml) was measured in milliliters using 5 ml calibrated collecting tube. Sperm-cell concentration was assessed with a haemocytometer. Hydrogen-ion concentration (pH) was measured using a pH cooperative paper ranging from 0 to 14 with 1 grade (Merck KgaA, 64271 Darmstadt, Germany).

The 2nd experiment examined the effect of the different levels of LT and CB by-products on the previous sperm quality parameters (% of motile, dead, abnormalities and sperm acrosomal damage) of extended rabbit semen during storage at 5^{o} C for 0, 24, 48, and 72 hours in LYC extender. Sperm quality parameters were evaluated as previously described in 1st experiment. The enzymes activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in the seminal plasma were measured after 0, 24, 48

and 72 hours of storage using commercial kits (bio-diagnostic company, EL-Doki, Giza, Egypt) according to the procedure outlined by the manufacturer.

***Statistical analysis*:**

The data of fresh semen (1st experiment) including, sperm motility, viability, abnormalities, acrosomal damage, sperm-cell concentration, semen ejaculate volume, and hydrogen-ion concentration were statistically analyzed as a completely randomize design with the Statistical Analysis System (SAS Institute, version 6.12, 1996) according to the following Model 1: $Y_{ij} = \mu + S_i + e_{ij}$, where Y_{ij} = an observation, μ = the overall mean, S_i = the fixed effect of i^{th} treatment (i= 1,2, .., 7), and e_{ij} = random error. The data of cooled semen (2nd experiment) including, sperm motility, viability, abnormalities, acrosomal damage and the enzymes activities of AST and ALT were statistically analyzed as a factorial arrangement (4 by-product levels x 4 storage periods), according to the following model: $Y_{ijk} = \mu + T_i + S_j + T_iS_j + e_{ijk}$ where Y_{ijk} = an observation, μ = the overall mean, T_i = the fixed effect of i^{th} treatment (i= 0,1, .., 3), S_j = the fixed effect of j^{th} storage time effect (j= 0, 24, 48, and 72 h), T_iS_j = the interaction effect, and e_{ijk} = random error. The significant differences were set at 0.05 probability level and tested using Duncan's multiple range tests (Duncan, 1955). Simple linear regression analysis (regression coefficient: b) was conducted using (SAS, 1996) to assess the rate of change (increase or decrease) in sperm quality parameters and the enzymes activities of AST and ALT during storage time.

RESULTS

Sperm quality parameters as affected by LT and CB by products:

In the 1st expirment (fresh semen). the results showed that semen ejaculate volume, percentages of motile, dead, abnormalities and sperm concentration were significantly improved ($P<0.05$ or $P<0.01$) in LT by-products supplemented groups compared with the control group (Table 2). However, hydrogen-ion concentration (pH) and the percentage of acrosomal damage sperm were not affected in bucks fed diets supplemented with LT by-product. Results in Table 2 showed that sperm concentration and sperm motility % increased gradually ($P<0.01$) with increasing the level of LT by-product supplementation. Interestingly, the lowest values of the percentages of dead and abnormal spermatozoa were recorded in rabbits group fed diet supplemented with 3% LT compared with control group (Table 2). Also, the highest values of sperm motility and sperm concentration were recorded in bucks fed diet supplemented with 3% LT (Table 2).

Table 2: Sperm quality parameters of adult male rabbits as affected by dietary LT and CB by-products supplementation

Treatment	Sperm quality parameters						
	Semen-ejaculate volume (ml)	Seminal pH	Sperm motility (%)	Dead sperm (%)	Sperm abnormalities (%)	Acrosomal damage (%)	Sperm concentration ($n \times 10^6$ mL)
Control	0.63^{b}	6.76	73.31^{b}	13.61^{a}	12.61^{a}	7.12	232.13^{d}
LT levels							
1%	0.75^{ab}	6.91	72.67^{b}	12.32^{ab}	10.71^{ab}	6.63	260.53^{b}
2%	0.70^{b}	6.75	75.66^{b}	10.53^{b}	9.26^{bc}	6.44	261.12^{ab}
3%	0.98^{a}	6.72	83.76^{a}	9.55^{b}	7.86^{c}	5.93	267.15^{a}
CB levels							
1%	0.69^{b}	6.81	72.38^{b}	12.21^{ab}	11.35^{ab}	7.27	248.86^{c}
2%	0.71^{b}	6.78	76.25^{b}	14.34^{a}	12.93^{a}	8.48	250.53^{c}
3%	0.78^{ab}	6.89	77.23^{b}	12.92^{ab}	8.16^{c}	6.74	248.41^{c}
SEM	**0.29**	**0.208**	**8.41**	**3.66**	**3.79**	**2.56**	**7.59**
P-value	**0.043**	**0.105**	**0.003**	**0.021**	**0.039**	**0.231**	**0.001**

Means in the same column with different letters differ significantly ($P<0.05$).
All values are least squares means. SEM: Standard error of mean.

With respect to CB by-product, the result of the 1st experiment showed that the CB by-product caused significant improvements (P<0.05 or P<0.001; Table 2) in % of sperm abnormalities and sperm concentration in fresh samples. The other semen quality parameters such as motility, viability, acrosomal damage as well as semen ejaculate volume and seminal pH were not significantly affected (P>0.05; Table 2) by CB by-product treatment.

In the 2nd experiment, results of cooled semen showed that the percentages of motile and dead sperm significantly improved (P<0.01, Table 3) in LT by-products supplemented groups compared with the control group. However, percentages of abnormalities and acrosomal damage sperm increased (P<0.01, Table 3) in groups fed diet supplemented with 1% and 2% LT by-product.

Additionally, the results of the 2nd experiment showed that, the percentages of motile and dead spermatozoa improved significantly (P<0.001; Table 4) only for rabbit bucks fed diet supplemented with 3% CB by-product. Also, the percentage of acrosomal damage sperm decreased significantly (P<0.001; Table 4) only for rabbit bucks fed diet supplemented with 1% CB by-product.

***Semen quality parameters as affected by storage time*:**

The effect of storage time on semen quality parameters of rabbit bucks fed on diets supplemented with different levels of LT or CB by-products during storage at 5°C up to 72 hours in LYC extender are presented in Tables 3 and 4. As shown in Tables 3 and 4, the percentages of motile sperm decreased gradually (P<0.01) over time. Moreover, the other sperm quality parameters such as the percentages of dead, abnormal sperm and acrosomal damage sperm increased gradually (P<0.01) over time for rabbit bucks fed diet supplemented with different levels of LT or CB by-products. Moreover, the present results showed that the alteration in semen quality parameters over time was more intense at the period from 48 to 72 hours than the others periods. For example, percentages of sperm motility decreased by about 27 % during the first 48 hours of storage (Table 3); while the reduction was about 29 % during the last 24 hours of storage (Table 3). This trend was also observed for the other sperm quality parameters (i.e., % of dead, abnormalities and acrosomal damage sperm; Tables 3 and 4) in rabbit bucks fed with different levels of LT or CB by-products. In addition, the effect of interaction between storage time and the different levels of LT by-product on all semen quality parameters was not significant (Table 3); however, the effect of interaction between storage time and the different levels of CB by-product was significant only on sperm abnormality and acrosomal damage (Table 4; P<0.01). This effect was reflected

Table 3: Sperm quality parameters of cooled rabbit semen as affected by lettuce by-product dietary supplementation and storage time.

Treatment	Sperm quality parameters			
	Motility %	**Dead %**	**Abnormal sperm %**	**Acrosomal damage %**
Lettuce level effect (%)				
0	51.61^{c}	29.44^{a}	22.12^{b}	15.36^{b}
1	54.01^{ab}	26.65^{b}	25.16^{a}	15.91^{b}
2	53.35^{bc}	28.55^{ab}	25.33^{a}	17.15^{a}
3	55.42^{a}	24.38^{c}	23.91^{ab}	15.01^{b}
P-value	0.001	0.002	0.007	0.028
Storage time effect				
0	80.02^{a}	12.02^{d}	10.17^{d}	7.06^{d}
24	70.92^{b}	15.66^{c}	13.83^{c}	9.55^{c}
48	52.67^{c}	26.53^{b}	22.85^{b}	14.12^{b}
72	24.33^{d}	46.11^{a}	43.13^{a}	30.47^{a}
P-value	0.005	0.001	0.001	0.002
Interaction between lettuce by-product and storage time				
P-value	**0.627**	**0.465**	**0.162**	**0.927**
SEM	**6.285**	**6.623**	**6.347**	**3.485**

Means in the same column with different letters differ significantly (P<0.05)
All values are least squares means. SEM: Standard error of mean.

in a similar trend of higher percentages of sperm abnormality and acrosomal damage at 72 hours than the storage times (Table 4).

In rabbit bucks fed with LT by-products, regression analysis showed a significant rate of decrease of 17.3 % per day in sperm motility (b = -17.3, P < 0.0001, Table 6). Also, regression analysis showed a significant rate of increase of 10.7 %, 9.0 % and 7.2 % per day in dead sperm, abnormal sperm, and acrosomal damage sperm, respectively. Furthermore, in rabbit bucks fed with CB by-products, regression analysis showed a significant rate of decrease of 17.2 % per day in sperm motility (b = -17.2, P < 0.0001). Moreover, the rate of decrease in dead sperm, abnormal sperm, and acrosomal damage sperm was 9.1, 7.6 and 5.9 % per day, respectively (Table 6).

***Activity* of *transaminase* enzymes as affected by LT and CB by products:**

The present results (Table 5) showed that ALT and AST levels in the seminal plasma were significantly (P<0.01) lower in all groups of rabbit bucks supplemented with the different levels of LT by- product compared to the

Table 4: Sperm quality parameters of cooled rabbit semen as affected by cabbage by-product dietary supplementation and storage time.

Treatment	Sperm quality parameters			
	Motility %	**Dead %**	**Abnormal sperm %**	**Acrosomal damage %**
Cabbage level effect (%)				
0	51.62^{b}	29.49^{a}	22.12^{ab}	15.35^{a}
1	52.55^{ab}	27.67^{a}	24.56^{a}	12.99^{b}
2	50.24^{b}	28.56^{a}	23.35^{a}	16.76^{a}
3	54.46^{a}	23.77^{b}	19.51^{b}	14.22^{ab}
P-value	**0.001**	**0.004**	**0.001**	**0.001**
Storage time (hours)				
0	80.02^{a}	12.02^{d}	10.17^{d}	7.06^{d}
24	70.94^{b}	16.78^{c}	12.95^{c}	9.84^{c}
28	58.61^{c}	29.82^{b}	20.51^{b}	13.99^{b}
72	28.11^{d}	49.51^{a}	44.75^{a}	32.01^{a}
P-value	**0.001**	**0.002**	**0.001**	**0.001**
Interaction between cabbage by-product and storage time				
0 x 0	77.67	13.81	10.61^{fh}	7.06^{hL}
0 x 24	69.33	18.65	14.26^{e}	9.93^{gf}
0 x 48	52.65	29.07	20.53^{d}	14.13^{e}
0 x 72	23.01	48.13	36.55^{b}	29.21^{c}
1 x 0	78.67	12.27	11.01^{f}	8.04^{gh}
1 x 24	69.11	18.26	15.87^{e}	10.73^{f}
1 x 48	53.65	23.01	26.73^{c}	14.21^{e}
1 x 72	25.34	49.15	47.18^{a}	33.26^{b}
2 x 0	79.76	13.13	10.63^{hl}	9.66^{gf}
2 x 24	68.15	16.53	12.41^{ef}	11.37^{f}
2 x 48	54.35	27.41	27.61^{c}	16.13^{d}
2 x 72	24.76	47.12	45.19^{a}	30.25^{c}
3 x 0	76.32	10.66	10.16^{fh}	6.21^{L}
3 x 24	71.11	13.65	11.46^{f}	10.35^{fg}
3 x 48	54.23	26.85	24.86^{c}	14.22^{e}
3 x 72	28.16	45.93	46.32^{a}	35.29^{a}
P-value	**0.451**	**0.149**	**0.002**	**0.003**
SEM	**5.583**	**6.124**	**5.536**	**4.297**

Means in the same column with different letters differ significantly (P<0.05)
All values are least squares means. SEM: Standard error of mean.

Table 5: The enzyme activities in seminal plasma of male rabbits as affected by lettuce and cabbage by-products dietary supplementation and storage time.

Treatment	Seminal plasma enzymes			
	Lettuce by-product		Cabbage by-product	
	ALT	AST	ALT	AST
By-product level effect (%)				
0	18.02^{a}	55.44^{ab}	18.03^{a}	55.44^{c}
1	16.42^{b}	52.53^{c}	16.36^{c}	57.16^{bc}
2	16.71^{b}	54.39^{b}	17.75^{ab}	58.09^{b}
3	16.25^{b}	56.21^{a}	17.43^{b}	60.92^{a}
P-value	**0.001**	**0.003**	**0.001**	**0.008**
Storage time effect (hours)				
0	10.44^{d}	34.83^{d}	11.09^{d}	40.45^{d}
24	12.77^{c}	43.33^{c}	13.21^{c}	46.31^{c}
48	16.22^{b}	51.23^{b}	16.25^{b}	54.12^{b}
72	25.38^{a}	78.39^{a}	25.76^{a}	81.63^{a}
P-value	**0.005**	**0.001**	**0.001**	**0.001**
Interaction between by-product and storage time				
0 x 0	11.05^{fg}	38.07^{e}	11.05^{hg}	38.07
0 x 24	14.16^{de}	44.81^{cd}	14.16^{f}	44.81
0 x 48	17.77^{c}	52.74^{b}	17.77^{d}	52.74
0 x 72	26.42^{a}	79.36^{a}	26.42^{ab}	79.36
1 x 0	9.75^{h}	32.75^{f}	10.83^{h}	36.44
1 x 24	12.18^{f}	40.52^{d}	12.95^{g}	45.48
1 x 48	16.09^{cd}	47.81^{c}	15.64^{ef}	51.93
1 x 72	24.48^{a}	77.59^{a}	23.31^{c}	81.34
2 x 0	10.21^{gh}	34.58^{f}	10.39^{h}	42.78
2 x 24	12.86^{f}	41.45^{d}	11.62^{hg}	45.36
2 x 48	15.06^{d}	49.21^{bc}	17.58^{d}	54.03
2 x 72	25.53^{ab}	79.21^{a}	27.75^{a}	82.19
3 x 0	10.86^{gh}	33.94^{f}	12.13^{g}	42.47
3 x 24	11.83^{fg}	46.57^{c}	14.05^{f}	46.55
3 x 48	16.03^{cd}	54.59^{b}	15.09^{ef}	57.09
3 x 72	24.75^{b}	77.41^{a}	25.26^{b}	83.85
P-value	**0.014**	**0.004**	**0.001**	**0.841**
SEM	**0.647**	**2.155**	**0.720**	**3.217**

Means in the same column with different letters differ significantly (P<0.01)
All values are least squares means. SEM: Standard error of mean
ALT: Alanine aminotransferase; AST: Aspartate aminotransferase

control. Similarly, ALT levels were significantly (P<0.01, Table 5) lower in rabbit bucks groups supplemented with the different levels of CB by-product compared to the control. However, AST levels were significantly higher (P<0.01, Table 5) in rabbit bucks supplemented with CB by-product compared to the control.

***Activity* of *transaminase* enzymes as affected by storage time:**

In the present study, the transaminase enzyme activities were measured during preservation of rabbit semen at low temperature (5°C). Results showed that AST and ALT activity was affected by the duration of storage in LYC extender (P<0.01). Results in Table 5, showed that AST and ALT activity increased gradually (P<0.01) over storage time for both treated and control samples. The effect of interaction between storage time and the treatments (different levels of LT and CB by-products supplementations) on the transaminase enzyme activities was significant (P<0.01; Table 5); except for AST levels in CB by-product samples were not significantly affected by this interaction (P>0.05; Table 5).

Additionally, in rabbit bucks fed with LT by-products, regression analysis showed a significant (P<0.0001, Table 6) rate of increase of 3.6 IU and 9.8 IU per day in ALT and AST levels, respectively. Similarly, in bucks fed with LT by-products, regression analysis showed a significant rate of increase of 4.8 IU/L and 13.7 IU per day in ALT and AST levels, respectively (Table 6).

DISCUSSION

***Semen quality parameters as affected by LT and CB by products*:**

As indicated in the present results, semen ejaculate volume, sperm motility, dead, abnormalities and sperm concentration were significantly improved in LT by-products supplemented groups compared with the control group. Present results agree with the findings of Alwan (2019) who showed significant improvement of sperm parameters including sperm motility, viability, abnormality and concentrations of the rabbits fed head lettuce (*Lactuca sativa*) meal compared to those fed alfalfa meal. Moreover, Abdel-Azeem *et al*. (2018) found that diet contained a mix of 0.5% rocket seeds (*Eruca Sativa*) and 0.5% carrot seeds (*Daucus Carota L*) increased rabbit ejaculate volume, sperm concentration, individual and total sperm motility, and live sperm and decreased abnormal sperm. Also, Ansari and Ganaie (2014) found that oral administration of aqueous extract of rocket leaves (*Eruca sativa*) for 8 weeks to male diabetic rats increased sperm quality parameters (*i.e*. sperm motility, viability and concentrations), endogenous antioxidant (glutathione) levels, serum testosterone and testicular weight; while decreased

Table 6. Regression coefficient (b ± standard error) of sperm quality parameters and seminal plasma enzymes on storage time (days), in lettuce and cabbage by-product treatments.

Items	b ± SE	P value
Lettuce by-product		
Motility %	-17.34 ± 0.38	<.0001
Dead sperm %	10.72 ± 0.43	<.0001
Abnormal sperm %	9.04 ± 0.20	<.0001
Acrosomal damage %	7.23 ± 0.25	<.0001
Alanine aminotransferase	3.64 ± 0.16	<.0001
Aspartate aminotransferase	9.78 ± 0.53	<.0001
Cabbage by-product :		
Motility %	-17.2 ± 0.40	<.0001
Dead sperm %	9.1 ± 0.38	<.0001
Abnormal sperm %	7.6 ± 0.31	<.0001
Acrosomal damage %	5.9 ± 0.26	<.0001
Alanine aminotransferase	4.8 ± 0.27	<.0001
Aspartate aminotransferase	13.7 ± 0.79	<.0001

sperm abnormality and lipid peroxidation. In human, Soubry *et al.* (2021) showed positive effect of consumption of vegetables and lettuce on semen quality (semen volume and sperm motility). Additionally, serum levels of testosterone were significantly increased in rats treated with extract of lettuce seed (Ahangarpour *et al*., 2014). In this respect, positive correlations between seminal plasma testosterone and sperm motility, viability and membrane integrity in frozen-thawed semen of Arabian horse were detected (El-Badry *et al*., 2016). It was decumented that, the natural antioxidant phytochemicals such as quercetin, rutin, apigenin and luteolin detected in lettuce and fresh vegetables break the chain reactions of free radicals responsible for peroxidation of lipids (Patil *et al*., 2009; Jideani *et al*., 2021). These antioxidants have the ability to maintain the sperm membrane fluidity and integrity by protecting sperm membrane against oxidative damage (Mazzi *et al*., 2011).

Furthermore, results of current study showed that, the % of sperm abnormalities, sperm concentration (in fresh semen), the % of motile and dead sperm (in cooled semen) were significantly improved in CB by-products supplemented groups compared with the control group. Our results are in partial agreement with those of other authors who showed that Lepidium

Meyenii (Maca), a traditional Peruvian cruciferous vegetable, belongs to the Brassicaceae family increases the sperm motility and sperm number in normal men without affecting serum level of testosterone, LH and FSH (Gonzales *et al*., 2004). Moreover, oral administration of an aqueous extract of Maca roots resulted in an improvement in spermatogenesis in adult male rats by acting on the initial stages of spermatogenesis (Gonzales *et al*., 2001). Furthermore, Tafuri *et al*. (2018) demonstrated that diet supplementation with Maca increased semen production and quality of stallions.

Previous investigations have shown that cabbage family contains many natural plant pigments (i.e., beta-carotene, lutein, zeaxanthin, and carotenoids) which exhibit strong antioxidant properties and may reduce the risk of some types of cancer (Michael *et al*., 1999; Onwuka *et al*., 2010; Adeoye *et al*., 2019a; Jideani *et al*., 2021). In this respect, Onwuka *et al*. (2010) studied the effect of cabbage on cadmium (Cd) –induced toxicity in Wistar rats. The authors showed that Cd increased significantly the levels of testicular and kidney malondialdehyde (MDA; a well-established indicator of oxidative stress) and reduced the testicular and kidney weight; but dietary supplemented with cabbage seem to have positive effects against Cd -induced changes in MDA levels and the testicular and kidney weight by decreasing Cd –associated oxidative stress. Sulforaphane (SFN) is a natural and highly effective antioxidant found in a large number of cruciferous plants such as cabbage, cauliflower, and broccoli. Also, Adeoye *et al*. (2019a; b) showed that the aqueous extract of white cabbage had potential of reducing liver oxidative stress in pre-diabetes induced male rats. Yang *et al.* (2016) studied the protective effect of SFN against Cd-induced oxidative damage in the testes of mice. The authors found that SFN treatment increased the levels of serum testosterone, sperm motility and total sperm count compared with the Cd-treated mice; while sperm abnormalities were decreased. Similarly, di-N-butylphthalate (DBP) is a ubiquitous environmental pollutant used in cosmetics products and plastic coating. DBP has toxic effects on body health, particularly on the male reproductive tract. Jiang *et al*. (2017) investigated the protective role of SFN (a natural antioxidant found in cabbage) against DBP-induced reproductive system damage in male mice. The authors showed that SFN supplementation significantly improved sperm count and motility, plasma and testicular testosterone levels, and testicular weight; while SFN reduced sperm abnormalities, oxidative stress, and testicular cell apoptosis. Additionally, Ogunlade *et al*. (2020) investigated the role of SFN on Aluminum trichloride ($AlCl_3$) induced testicular toxicity in adult male Wistar rats. The authors showed ameliorative effect of SFN on $AlCl_3$ -induced testicular toxicity by increasing the levels of antioxidant enzymes (superoxide dismutases,

glutathione, catalase) with corresponding decrease in lipid peroxidation. Also, Ogunlade *et al*. (2020) showed a decrease in sperm motility, viability and count after exposure to $AlCl_3$. However, the combined administration of SFN and $AlCl_3$ improved the previous sperm quality parameters.

Information about the direct effect of LT and CB by-products supplementation on sperm quality parameters of rabbit bucks is not available, so it is difficult to compare our findings. We speculated that the improvements observed in sperm quality parameters (i.e. sperm motility, viability, abnormalities and sperm concentration) of bucks fed with LT and CB by-products supplementation could be attributed to the different types of natural antioxidants found in LT and CB.

Semen quality parameters as affected by storage time:

Storage time induced a significant impairment in all parameters of sperm quality measured from 0 to 72 hours of storage at 5°C. These results agree with other studies on rabbit (El-Kelawy *et al*., 2012; El-Seadawy *et al*., 2017), ram (Mohamed *et al*., 2019), goat (Lima *et al*., 2013; Liu *et al*., 2019) and bull sperm (Mohamed *et al*., 2019). Recently, Barranco *et al*. (2019) found that sperm motility decreased as storage time increased (up to 144 hours), while sperm viability was not affected in boar semen. Moreover, Liu *et al*. (2019) observed the detrimental effect of goat spermatozoa storage at 5°C in other sperm quality parameters, showing an increase in apoptosis and defects of mitochondria as the length of sperm storage time increased. The obvious reduction in sperm quality parameters detected in the present study during storage time may be due to many factors, such as the partial loss of sperm membranes integrity and functions that results in the loss of vital cell components, which consequently decreased sperm metabolic rate, motility and viability (De Pauw *et al*., 2003; Lima *et al*., 2013). Also, an increase in lactic acid formation due to anaerobic sperm metabolism can alter the osmotic pressure and the pH of the media, which consequently affect negatively sperm function (El-Kelawy *et al*., 2012; Lima *et al*., 2013). Cooling induced reductions in sperm function may also be due to oxidative damage induced by the deleterious effects of reactive oxygen species and membrane lipid peroxidation (Peris *et al*., 2007; Aitken *et al*., 2014; Barranco *et al*., 2019). In this context, Peris *et al*. (2007) showed that sperm motility appears to be a more susceptible to oxidative stress than other sperm parameters such as sperm viability, capacitation, and sperm chromatin stability. The mechanism by which sperm motility is lost when exposed to oxidative stress is not exactly known. However, both oxidative damage to the axoneme and reduction in intracellular adenosine triphosphate (ATP) levels seem to be involved (Aitken *et al*., 2014).

***Activity* of *transaminase* enzymes as affected by LT and CB by products:**

The activity of transaminase enzymes (AST-ALT) in semen is a good indicator of semen quality because these enzymes measure the stability of sperm membrane (Khan *et al*., 2012). In general, the low activity of AST and ALT observed in most supplemented groups might have some positive effects on sperm function (Khan *et al*., 2012; Feng *et al*., 2015). Moreover, reduce in AST and ALT activities of seminal plasma in rabbit groups supplemented with the different levels of LT or CB by-products suggest that it maintains structural stability of the sperm. In this context, Abdel-Azeem *et al*. (2018) reported that, seminal plasma AST and ALT in rabbits' bucks decreased with diets supplemented with 0.5 % or 1.0 % rocket seeds (*Eruca Sativa*), carrot seeds (*Daucus Carota L*) and bay laurel leaves (*Bay laurel Nobilis L*) and their mixture as compared to the control group. To our knowledge, little or no published reports were focused on the effect of LT and /or CB by-products supplementation in diets on the activity of AST and ALT in rabbit semen stored at 5°C.

***Activity* of *transaminase* enzymes as affected by storage time:**

As expected, our results showed that AST and ALT activity increased gradually ($P<0.01$) over storage time for both treated and control samples. Present results are in agreement with the results of other studies on camel (Zeidan *et al*., 2008), ram (Tejaswi *et al*., 2016; Mohamed *et al*., 2019) and cattle (Divya and Jayavardhanan, 2014; Mohamed *et al*., 2019). Zeidan *et al*. (2008) demonstrated that prolongation of storage time up to 3 days at 5°C increased the leakage of AST and ALT enzymes gradually into the extracellular medium of the extended cooled camel semen.

The significant effect of interaction between storage time and the treatments on the transaminase enzyme activities suggest that, feeding bucks on rations supplemented with different levels of LT and CB by-products can improve sperm membrane integrity during the storage at 5°C up to 72 hours (as indicated by the low levels of seminal plasma activities of AST and ALT in treated bucks compared to control). Therefore, one should bear in mind that sperm quality during liquid storage at 5°C is a function of storage time and nutritional status of rabbit bucks.

As discussed earlier, our results showed that the percentages of dead and abnormal sperm increased gradually ($P<0.01$) over storage time in both control and treated samples, which in turn may affect sperm membrane stability negatively. Consequently, high levels of AST and ALT enzymes in the extra cellular fluid due to sperm membrane instability and ease of leakage of these enzymes from sperm (Divya and Jayavardhanan, 2014). Transaminase enzyme activities (AST-ALT) are important for sperm metabolic processes which

provide energy for motility, viability and fertility of spermatozoa (Perumal *et al*., 2013; Tejaswi *et al*., 2016). Moreover, negative correlations between ALT and AST enzymes and sperm quality parameters have been reported in rabbits (Yousef *et al*., 2003) and poultry (Khan *et al*., 2012).

In conclusion, exploring a new feeding strategy is an important issue to improve reproductive performance in rabbits. Addationaly, using agricultural by-products in animal feeds can help farmers reduce feed costs, also, to minimize the risk of environmental population caused by the agricultural waste. The results of present study clearly demonstrate that dietary supplementation with LT or CB by-products enhanced the reproductive performance in rabbit bucks. This was manifested by the higher ejaculate volume, sperm concentration, sperm motility, as well as the lower percentages of dead, abnormalities, and sperm with acrosomal damage that observed in the semen of treated bucks compared to their control. Storage time induced a significant impairment in all parameters of semen quality measured during 72 hours at 5°C. Moreover, reduce in AST and ALT activities of seminal plasma in rabbit groups supplemented with the LT or CB by-products suggest that it maintains structural stability of the sperm. It could be recommended to supplement LT and CB by-products at the level of 3% rather than 1%, and 2 %, to the diet of rabbits, since the dietary supplementation at a level of 3% had better effects on semen quality parameters in fresh and cooled rabbit semen.

Conflict of interest: All authors certify no conflict of interests.

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تأثير المكملات الغذائية من الخس والكرنب كمنتجات ثانوية على السائل المنوي الطازج والمبرد للأرانب

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استخدام المنتجات الثانوية فى تغذية الحيوانات يمكن أن يساعد المربين فى تخفيض تكاليف التغذية وتكاليف التخلص من هذه المخلفات ، كما يقلل من المشاكل البيئية المرتبطة بهذه المخلفات. استخدم في هذه التجربة اثنان وأربعون ذكر أرنب نيوزيلاندي ابيض عمر 8 شهور، تم تقسيمهم عشوائيا إلى 7 مجاميع. المجموعة الأولى (كنترول) غذيت على عليقة أساسية. المجموعات الثانيه والثالثة والرابعة غذيت على العليقة الأساسية مضاف اليها 1، 2، 3% مخلفات ثانويه للخس المجفف. المجاميع الخامسة والسادسة والسابعة غذيت على العليقة الاساسية مضاف اليها 1، 2، 3 % مخلفات ثانوية للكرنب المجفف. تم دراسة تجربتين.

التجربه 1 لدراسة تاثير اضافه الخس والكرنب للعليقه الأساسية وتاثيره على مقاييس جودة الحيوانات المنوية فى السائل المنوى الطازج والتى تشمل (الحجم ، الحركة ، الحيوية ، الشواذ ، ضرر الاكروسوم و تركيز الحيونات المنوية).

التجربة 2 لدرسة تاثير اضافه الخس والكرنب وكذلك تاثير مدة التخزين على معايير جوده الحيوان المنوى السابقة فى السائل المنوى المبرد على درجة حرارة 5 مئوى لمده 72 ساعه. الأنشطة الأنزيمية لــ اسبرتات امينو ترانسفيراز (AST) و الانين امينوترانسفيريز (ALT) تم قياسها.

اظهرت النتائج فى التجربه 1، ان تركيز الحيوانات المنوية زادت فى كل المجاميع المضاف اليها الخس او الكرنب. كما أن نسبة الميت والشاذ انخفضت. حجم القذفة والحركة زادت فى المجموعة المضاف اليها 3% خس. في التجربة 2 أظهرت أن نسبة حركة الحيوانات المنوية زادت ونسبه الميت انخفضت فى الذكور المعاملة 1 او3 % خس مقارنه بالكنترول. أيضا إضافه الكرنب أدى لزيادة نسبة حركة الحيوانات المنوية وخفض نسبة الميت وضرر الاكروسوم مقارنة بالكنترول. أظهرت النتائج أن مستوى (AST و ALT) كان منخفض فى المجاميع المضاف إليها الخس أوالكرنب مقارنه بالكنترول.

التوصية: من النتائج الحالية أوضحت أن أعلى أداء تناسلي أمكن تحقيقه عندما غديت ذكور الأرانب على عليقة مضاف إليها 3% خس أو كرنب كمنتج ثانوى.