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SEASONAL EFFECTS ON SEMEN QUALITY IN BLACK BALADI AND WHITE NEW ZEALAND RABBIT BUCKS

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Abstract: A total of 32 sexually mature rabbit bucks (at 6th month of age) were used in this experiment to study the effects of breed (Black Baladi -BB- *vs*. White New Zealand -WNZ-) and season (summer *vs*. winter) on libido and seminal parameters. The experimental design was completely random with four groups arranged factorially (two breeds and two seasons) with eight rabbits in each group and three months per season. The results obtained from this study indicated that, libido (14.5 *vs*. 21.9 sec) and physical semen characteristics represented by the volume of semen per ejaculate without gel fractions (0.70 *vs*. 0.49 mL), sperm-cell concentration (703 *vs*. 597×10^6/mL), total sperm output (513 *vs*. 293×10^6/ejaculate), sperm abnormalities (11.6 *vs*. 14.0%), acrosomal damages (8.6 *vs*. 11.5%), dead spermatozoa (13.9 *vs*. 16.0%), and advanced sperm motility (63.2 *vs*. 57.1%) were significantly (P<0.01) better on BB rabbit bucks than on WNZ breed. In addition, these parameters proved to be significantly (P<0.01) better in winter season (15.8 sec, 0.68 mL, 702×10^6/mL, 487×10^6/ejaculate, 10.7%, 8.4%, 12.7%, and 65.6%, respectively) than in summer (20.6 sec, 0.52 mL, 598×10^6/mL, 319×10^6/ejaculate, 15.0%, 11.7%, 17.3%, and 54.7%, respectively). Following the hypo-osmotic swelling test (HOST) at 75 mOsmol/L during incubation at 37°C for 20 min, the percentages of sperm motility, swollen spermatozoa, and spermatozoa with coiled tails were higher for BB bucks (16.98, 44.08, and 39.13) than for the WNZ breed (7.2, 32.3, and 26.0). This was also the case in the winter season (14.4, 42.7, and 38.5) when compared to summer (9.8, 33.7, and 26.6), respectively. We concluded that, under Egyptian conditions, both libido and semen quality in BB bucks seems to be better than those displayed by the WNZ bucks. Nevertheless, overall semen characteristics were better in winter than in the summer season.

Key words: rabbit, semen characteristics, season, breed.

INTRODUCTION

Following the appearance of avian influenza virus in many countries including Egypt and the reduction in white meat production from poultry, an opportunity has arisen for the rabbit industry to play an important role in solving, at least in part, this meat shortage by overcoming the gap between animal protein demand and supply (Seleem *et al*., 2007). Black Baladi (BB) rabbits, an Egyptian meat-type breed, were originated by crossing heavy Baladi does with pure Giant Flander bucks, through the selection of heavy body weight and pure black colour traits over several generations (Meshreky *et al*., 2005). BB rabbits are characterized by their high tolerance to climatic stress and their resistance to disease (Khalil, 2002).

Over the last two decades some new rabbit breeds were imported into Egypt (e.g. White New Zealand -WNZ- breed) with the purpose of commencing commercial-scale rabbit production on an intensive level (Farid *et al*., 2000). It seems that these breeds successfully adapted to the local environmental conditions, because the breeds are now widespread and are being reared in many rabbitries (Daader and Seleem, 1999; Enab *et al*., 2000). There is some evidence that the reproductive and productive performances of

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WNZ rabbits reared in Egypt were superior to that of other imported breeds with regards to semen quality and reproductive performance (Daader *et al*., 2002). However, up until now, there has been insufficient information about the overall performance potential of this breed. Moreover, information and comparative studies undertaken on the productive and reproductive performances of different exotic and native breeds of rabbits are still scarce and further investigations are required.

A great deal of effort and focus was aimed at introducing Artificial Insemination (AI) in the rabbit industry in both limited usage and wide scale rabbitries (El-Gaafary and Marai, 1994). In addition, the employment of AI maximizes the financial profit of the rabbitry by reducing the number of pen-raised bucks and, consequently, the number of non-productive cages (Alvariño, 2000, Khalifa *et al*., 2000; Seleem and Riad, 2005). When AI is applied in a rabbitry, it is estimated that one single buck may affect the fertility and prolificacy of about one hundred does (Seleem, 2005). Consequently, the reliable evaluation of both semen and the fertilizing ability of bucks are of vital importance to the success of the AI technique. However, unfortunately, there is no individual basic parameter exists for semen evaluation that can be employed as a reliable predictor of sperm fertilizing ability. As such, finding a laboratory test, capable of consistently predicting the fertility of a semen sample would be highly desirable (Carluccio *et al*., 2004; Lavara *et al*., 2005). The hypo-osmotic swelling test (HOST) was used to evaluate the response of human spermatozoa to hypo-osmotic conditions (Jeyendran *et al*., 1984; Zaneveld *et al*., 1990). Daader and Seleem (2005) have reported that in rabbits HOST is more reliable in assessing the outcome of in vitro fertilization than other semen parameters.

The present study was planned to evaluate both semen quality and the spermatozoa response to HOST of a local breed represented by BB and a foreign breed represented by WNZ rabbit bucks during the winter and summer seasons.

MATERIALS AND METHODS

Location and climatic data

This work was carried out in an industrial rabbitry − located at El-Obour, Qalubia, Egypt − during the winter (January to March) and summer seasons (July to September) of 2005. During the experimental period, the maximum and minimum air temperatures (^{0}C), relative humidity (RH%), and temperature-humidity index (THI) values were recorded inside the rabbitry on a monthly basis (Table 1). The THI was estimated using the formula reported by (Marai *et al*., 2002): THI=db^{0}C-[(0.31-0.31 (RH/100)) (db^{0}C-14.4)] where db ^{0}C represents the dry bulb temperature and RH is the relative humidity expressed as a percentage. The calculated values for the THI were classified as follows: values below 72 represented the absence of heat stress; from 72 to 83 moderate heat stress; from 83 to 86 severe heat stress, while values above 86 signified very severe heat stress.

Animals and managements

Thirty-two rabbit bucks at 6 months of age (sexually mature), 16 BB and 16 WNZ, were used in this study: eight rabbit bucks for each breed during summer, and another eight during winter. Animals were fed a commercial diet *ad libitum* (15.5% crude protein, 2.3% ether extract, 16% crude fibre, 2600 kcal ED/ kg) that satisfied the nutritional requirements of both the growth and mature phases of rabbits according to NRC (1994) recommendations. All animals were kept under the same management practices and hygienic conditions and were raised in wired batteries in a windowed rabbitry with natural ventilation. A constant supply of fresh tap water was automatically available via stainless steel nipples located inside each cage. The photoperiod was 13-14 h light per day during summer and 10-11 h light per day during the winter season.

Table 1: Means and standard error (mean±standar error) of maximum and minimum air temperatures (^{0}C), the percentage of relative humidity (%) and calculated values for the temperature-humidity index inside the rabbitry during the experimental period.

	Air temperature		Relative humidity		Temperature-humidity index	
Month	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum
January	22.5±0.9	13.1±0.6	42.0±1.9	38.8±1.7	67.9	54.9
February	24.5±1.3	14.3±0.7	42.2±2.1	23.0±1.5	70.4	57.9
March	25.3±1.6	18.3±1.3	55.8±1.6	35.0±1.2	72.8	62.6
July	33.8±1.3	23.0±1.5	86.0±1.7	64.6±2.1	90.1	70.4
August	34.4±0.7	24.1±1.1	76.1±2.1	76.0±1.9	89.2	72.2
September	31.2±1.5	23.0±1.3	89.1±2.8	30.0±0.6	86.4	67.4

Libido and semen

Libido (sexual desire) was measured in terms of reaction time in seconds and was estimated from the time the doe was placed inside the buck's cage up to the point when the buck started to mount the doe (Daader *et al*., 1999). Semen was artificially collected twice a week for up to 12 weeks per season using an artificial vagina as described by Moce *et al*. (2000). Ejaculated semen samples from each rabbit buck were evaluated individually after dilution (1:20) with a saline solution supplemented with egg yolk (0.9 mg NaCl, 5 mL egg yolk, 50000 IU sodium penicillin and 50000 µg streptomycin sulphate dissolved in 75 mL of sterilized distilled water). The ejaculate volume without gel fractions (mL) and the sperm-cell concentration ($\times 10^6$/mL) were measured using a graduated conical tube and a Thoma-Zeiss cell counting chamber, respectively. The total sperm output ($\times 10^6$/ejaculate) was then calculated. Sperm abnormalities (%) were estimated using a magnification of 500× with a differential interference contrast microscope. A Giemsa stain was used to measure the acrosomal damages (%) of spermatozoa with an abnormal apical ridge. Non-vital sperms were calculated as dead spermatozoa (%). The sperm motility grade scored (0-5 grade), and advanced sperm motility (%) viewed under a microscope with phase contrast optics (100×), were estimated according to Seleem (2005). Acrosome status was determined using a Giemsa stain procedure as described by Watson (1975).

HOST was determined in semen samples in a 1:10 dilution of hypo-osmotic solution (75 mOsmol/L; Moce *et al*., 2004). Final osmolarity of the test solutions, measured by the freezing point depression, was obtained via serial dilutions ranging from 300 mOsmol/L (normal osmolarity) to 75 mOsmol/L (hypo-osmolarity). The grades and percentages of sperm motility and spermatozoa with swollen heads and coiled tails were estimated at 37^{o}C at different intervals during the incubation periods (0, 10, and 20 min).

Statistical analysis

The experimental design was completely selected at random with four treatments arranged factorially with two breeds (BB *vs*. WNZ) and two seasons (summer *vs*. winter) with eight rabbits for each treatment and three months for each season. Pooled data regarding the main effects and interactions were evaluated by a variance analysis according to Snedecor and Cochran (1967) using the General Linear Model (GLM) procedure, SAS software, version 8.2 (SAS, 1997). This was done on a monthly basis. Percentage values were transformed using arcsine before being statistically analyzed. Duncan's multiple range test (Duncan, 1955) was used to test the significance of the differences between means.

RESULTS AND DISCUSSION

Climatic conditions

During the months of July, August, and September the maximum recorded air temperatures varied from 31.2 to 34.4°C and the maximum THI ranged from 86.4 to 90.1, indicating that rabbits were exposed to very severe heat stress during this season (Table 1). This concurs with the results of Marai *et al*. (2002) who stated that heat stress, responsible for impaired reproductive performance, occurs whenever the temperature is ≥27.8°C, and severe heat stress appears when the temperature is higher than 28.9°C or the THI greater than 83.

Libido and the physical quality of semen

As reported in Table 2, total sperm output was lower in summer than in winter as was expected, but the differential values for BB bucks (225.3×10^6/ejaculate) were double that of the WNZ bucks (110.1×10^6/ ejaculate). In contrast, the opposite was found for sperm abnormality (3.0 *vs*. 5.5%), acrosomal damages (2.00 *vs*. 4.87%), and dead sperms (3.33 *vs*. 6.00%) in BB *vs*. WNZ buck, respectively. These data seems to show that WNZ bucks had higher sensitivity to temperature changes than BB bucks. This could explain the significant interactions observed between the breed and season.

The results indicated that BB rabbit bucks performed significantly better than WNZ bucks in terms of libido (−7.4 sec) and semen characteristics or sperm production. This was represented by the ejaculate volume without gel fractions (+0.21 mL; $P<0.01$), sperm-cell concentration ($+106\times10^6$/mL; $P<0.01$), total sperm output ($+220\times10^6$/ejaculate; $P<0.01$), sperm abnormalities (−2.41%; $P<0.01$), acrosomal damages (−2.96%; $P<0.01$), dead spermatozoa (−2.12%; $P<0.01$), the sperm motility grade scored (+0.33 grades; $P<0.05$), and advanced sperm motility (+6.15%; $P<0.01$), respectively. These results are comparable with those obtained by Meshreky *et al*. (2005) who compared Red Baladi and V-line (line selected on the basis of litter size at weaning) rabbit bucks. Also, elsewhere similar values for the overall mean volume of ejaculate were reported (Vicente *et al*., 2000) in R-line rabbits (line selected on the basis of growth rate from weaning to slaughter (28-63 days of age)) and also for sperm abnormalities (García-Tomás *et al*., 2006) in two rabbit sire lines and their reciprocal crosses. However, values for sperm-cell concentration and motility were higher than those reported by the same authors. These differences could be explained not only by genetic and environmental factors, but also by the different criteria employed for the evaluation and the use of various semen processing technologies.

The libido and the physical quality of semen ejaculated by BB and WNZ rabbit bucks during the winter season (15.8 sec, 0.68 mL, 702.1×10^6/mL, 486.6×10^6/ejaculate, 10.71%, 8.40%, 12.65%, 3.92 grade, and 65.63%, respectively as above) were significantly ($P<0.01$) better than those observed in the summer season (20.6 sec, 0.52 mL, 597.7×10^6/mL, 318.9×10^6/ejaculate, 14.96%, 11.73%, 17.31%, 3.21 grade, and 54.69%, respectively as above). These results concur with those of Daader *et al*. (1997) and Seleem *et al*. (2007). Daader and Seleem (1999) and Seleem *et al*. (2006) reported the same trend in reproductive traits in WNZ rabbit bucks. It was established that semen volume increased and motility indexes decreased during summer (Roca *et al*., 2005). Also, Finzi *et al*. (1995) reported that the daily exposure of rabbits in a climatic chamber to high ambient temperature (30^0C) and humidity (70%) for 21 h over a 60 day period increased the number of abnormal spermatozoa.

Response of spermatozoa to HOST

Data presented in Table 3 shows that, the highest grades and percentages for progressive sperm motility at 75 mOsmol/L osmolarities, during incubation at 37°C for up to 20 min were recorded by BB semen. Black Baladi rabbit spermatozoa displayed a significantly higher resistance ($P<0.01$) than that of the WNZ

Table 2: Libido and semen characteristics in Black Baladi (BB) and White New Zealand (WNZ) rabbit bucks during summer and winter seasons[1].

		Libido (Sec.)	Semen ejaculate volume (mL)	Sperm-cell concentration ($\times 10^6$/mL)	Total sperm output ($\times 10^6$/ejaculate)	Abnormal Sperms (%)	Acrosomal damages (%)	Dead sperms (%)	Sperm motility grade (0-5)	Advanced sperm motility (%)
Overall mean (n=96)		18.2±0.54	0.60±0.019	649.9±12.6	402.7±19.0	12.83±0.36	10.06±0.33	14.98±0.39	3.56±0.078	60.16±1.17
Main effects (n=48)										
Breed:	BB	14.5	0.70	703.1	512.8	11.63	8.58	13.92	3.73	63.23
	WNZ	21.9	0.49	596.7	292.7	14.04	11.54	16.04	3.40	57.08
Season:	Summer	20.6	0.52	597.7	318.9	14.96	11.73	17.31	3.21	54.69
	Winter	15.8	0.68	702.1	486.6	10.71	8.40	12.65	3.92	65.63
Standard Error		0.43	0.018	14.3	17.6	0.36	0.34	0.40	0.096	1.41
Interaction (n=24)										
BB:	Summer	16.7	0.60	644.2	400.2^b	13.13^b	9.58^b	15.58^b	3.42	57.71
	Winter	12.3	0.80	762.1	625.5^a	10.13^c	7.58^c	12.25^c	4.04	68.75
WNZ:	Summer	24.5	0.43	551.3	237.6^c	16.79^a	13.88^a	19.04^a	3.00	51.67
	Winter	19.3	0.55	642.1	347.7^b	11.29^c	9.21^b	13.04^c	3.79	62.50
Standard Error		0.60	0.026	20.2	24.8	0.51	0.48	0.56	0.136	1.99
Significance[2]										
Breed		**	**	**	**	**	**	**	*	**
Season		**	**	**	**	**	**	**	**	**
Breed × Season		NS	NS	NS	*	*	**	*	NS	NS

[1]Means in the same column with different superscripts differ significantly (P<0.10).

[2]NS = not significant ($P > 0.10$); * $P < 0.05$; ** $P < 0.01$.

Table 3: Effect of the Hypo-Osmotic Swelling Test (HOST) on sperm motility grade (0-5), advanced sperm motility (%), swollen head (%) and coiled tail (%) in Black Baladi (BB) and White New Zealand (WNZ) rabbit bucks during summer and winter seasons.[1]

		Motility grade (0-5)			Advanced sperm motility (%)			Swollen head (%)			Coiled tail (%)		
Incubation time (min)		0.0	10.0	20.0	0.0	10.0	20.0	0.0	10.0	20.0	0.0	10.0	20.0
Overall mean (n=96)		3.51	1.75	0.78	56.46	31.62	12.08	2.74	22.96	38.20	1.89	18.52	32.54
(SE)		(0.080)	(0.071)	(0.061)	(1.00)	(1.47)	(0.83)	(14)	(0.76)	(0.99)	(0.13)	(0.86)	(1.08)
Main effects (n=48)													
Breeds:	BB	3.85	2.04	1.04	60.73	39.79	16.98	3.33	28.38	44.08	2.71	24.48	39.13
	WNZ	3.17	1.46	0.52	52.19	23.44	7.19	2.15	17.54	32.31	1.06	12.56	25.96
Seasons:	Summer	3.25	1.48	0.71	53.44	27.40	9.79	2.31	20.92	33.67	1.58	15.63	26.58
	Winter	3.77	2.02	0.85	59.48	35.83	14.38	3.17	25.00	42.73	2.19	21.42	38.50
Standard Error		0.093	0.082	0.079	1.21	1.62	0.87	0.17	0.69	0.89	0.13	0.75	0.83
Interaction (n=24)													
BB	Summer	3.46^{b}	1.67	0.96	56.46	35.21	13.54^{b}	2.71	25.88	38.25^{b}	2.50	21.25	33.42
	Winter	4.25^{a}	2.42	1.13	65.00	44.38	20.42^{a}	3.96	30.88	49.92^{a}	2.92	27.71	44.83
WNZ	Summer	3.04^{c}	1.29	0.46	50.42	19.58	6.04^{c}	1.92	15.96	29.08^{c}	0.67	10.00	19.75
	Winter	3.29^{bc}	1.63	0.58	53.96	27.29	8.33^{c}	2.38	19.13	35.54^{b}	1.46	15.13	32.17
Standard Error		0.132	0.116	0.111	1.71	2.29	1.22	0.24	0.97	1.26	0.18	1.06	1.18
Significance[2]													
Breed		**	**	**	**	**	**	**	**	**	**	**	**
Season		**	**	NS	**	**	**	**	**	**	**	**	**
Breed × Season		†	NS	NS	NS	NS	†	NS	NS	†	NS	NS	NS

[1]Motility grades scored from 0 (no motility) to 5 (the highest motility). Means in the same column with different superscripts differ significantly ($P<0.10$).
[2]NS = Not significant ($P>0.10$); † $P<0.10$; ** $P<0.01$

rabbits to the hypo-osmotic solution at 37°C for 20 min. This is represented by the progressive sperm motility grades scored (+0.52 grade), advanced sperm motility (+9.79%), swollen heads (+11.77%), and coiled tails (+13.17%), respectively. Also, the percentages of sperm motility, swollen heads, and coiled tails of BB and WNZ rabbit spermatozoa in hypo-osmotic solution at 37°C for 20 min were significantly ($P<0.01$) higher in winter (14.4, 42.7, and 38.5%) than those obtained during the summer season (9.8, 33.7, and 26.6%), respectively. When understanding the changes that spermatozoa are subjected to in a hypo-osmotic environment, if we accept the suggestion that these changes indicate a functionally intact plasma membrane, and that this is necessary for normal spermatozoal function, then, within a semen sample, the more cells with swollen plasma membranes, the better the potential sperm quality (Neild *et al*., 2000). In addition, it was evident that the advancement of the incubation time at 37^{0}C for up to 20 min decreased the grades and percentages of progressive sperm motility and increased the percentages of spermatozoa with swollen heads and coiled tails for all experimental groups with regards to main effects or interactions. These results are comparable with those obtained by Daader and Seleem (2005). A similar trend was previously recorded by Kumi-Diaka (1993) in canine spermatozoa, Correa and Zavos (1994) in bovine, Vazquez *et al*. (1997) in boar and Moussa (1999) in ram spermatozoa. The response of spermatozoa to hypo-osmotic solution may be due to the transportation of the physical and biochemical compounds across the sperm cell membrane which plays an essential role in the biochemical process and consequently in sperm viability and fertilizing capabilities (Zavos, 1983). Daader and Seleem (2005) added that the abrupt drop in osmotic pressure may result in malfunction in the physiological processes of spermatozoa. From these results is deduced that, under Egyptian sub-tropical conditions, the reproductive efficiency of BB seems to be greater than that of the WNZ rabbit bucks. Given that the hypo-osmotic condition that spermatozoa are subjected to is considered to be a stressful factor, it could be concluded that the response of rabbit spermatozoa to the HOST could be a good indicator of the reproductive ability of rabbit bucks. As such, where spermatozoa show better results for motility or survivability, this can be taken as a good indicator of their fertilizing ability. In such cases, it is only necessary to carry out one test in order to obtain an improved evaluation of spermatozoa.

From the results obtained from the present study it could be concluded that the semen characteristics of BB rabbits presented better results than those of the WNZ rabbits under environmental conditions in Egypt. This was also the case during the winter season in comparison with summer. Although WNZ rabbit bucks display qualitatively smaller values, almost constant values are maintained throughout the entire experimental period. Finally, it could also be concluded that the HOST seems to be a good indicator of the reproductive ability of rabbit bucks.

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