SEMEN QUALITY PARAMETERS OF ADULT MALE NZW RABBITS FED DIETS ADDED WITH TWO DIFFERENT TYPES OF PROBIOTICS

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SUMMARY

The present study was performed to evaluate the potential use of probiotics for improving semen quality of mature NZW rabbits. Twenty adult male NZW rabbits were assigned randomly into four equal groups that were fed four experimental diets for eight weeks. All experimental groups were fed a uniform rabbits pelleted diet, where (R1) diet without addition (control), (R2) diet added with 0.1 % *Bacillus subtilis*, (R3) diet added with 0.1 % live *Saccharomyces cerevisiae* and (R4) diet added with 0.05 % *Bacillus s.* +0.05 % *Saccharomyces c*. Semen samples were collected at 5th, 6th, 7th and 8th weeks of the experimental period from all tested rabbits to evaluate ejaculate volume, total sperm output, sperm cell concentration, advanced sperm motility, sperm abnormalities, dead spermatozoa, testosterone level, initial fructose concentration and reaction time(Libido).Results obtained revealed that adding probiotics to the diet of NEW rabbit bucks improved (P< 0.05) their productive capability of male rabbit. Physical semen characteristics, blood testosterone level and initial fructose as well as the reaction time (Libido) were improved with supplemented diets, however these improvements for all parameters were associated with feeding the diet supplemented with *Bacillus s.* + *Saccharomyces c*. mixture. Therefore, it can be concluded that adding diet of buck with mixed probiotics culture of *Bacillus s.* and *Saccharomyces c*. in a ratio of (50:50 %) improved semen quality and reproductive performance.

Keywords: *rabbits; probiotics; semen quality; reaction time.*

INTRODUCTION

Developing countries like Egypt are often characterized by animal protein deficiency. This is not only because of size of farm animal's populations, but it is mainly due to the low productive and reproductive capabilities of the local animals. The increase in animal protein production may come from short-life cycle animals kept by the small farmers such as rabbits. The farming of rabbits has been encouraged to minimize the gap between demand and supply of animal protein (F.A.O. 1987). Therefore, production of rabbits had gradually increased at the last two decades in Egypt to support meat production (Rashwan and Marei, 2001 and Seleem, 2003). Rabbit production in Egypt is currently difficult due to high feeding cost. This problem has limited establishing of new rabbitaries and expansion of this kind of project. Several investigations have carried out to reduce the high cost of rabbit feed.

Several attempts have been carried out to promote the rabbit production and reproduction by using probiotics (Osman, 2005 and Seleem *et al*., 2007a). Probiotics have been defined as microbial dietary supplements that enhance the growth of desirable gastrointestinal microbes which beneficially affect the health of animal host (Schrezenmeir and de Vrese, 2001). The direct nutritional benefits of probiotic include feed efficiency, enzymatic contribution to digestion, inhibition of pathogenic microorganisms, and growth promoting factors (Vershuere et al., 2000). Therefore, probiotics increased feed conversion efficiency, live weight gain and resistance to diseases in growing animals (Sissons, 1989). However, few reports have studied the effect of incorporating probiotics in male rabbit diets on semen quality.

Quality of semen is affected by many factors (health status, environmental conditions, diet, etc) which have received little scientific attention. Hence, this study aimed to evaluate bulks reproductive capability (semen quality and libido) as affected by probiotic addition (*Bacillus subtilis* or live *saccharomyces cerevisiea* alone or in a mixture 50:50 % of each).

MATERIAL AND METHODS

The present study was conducted at the Experimental Farm Station of the by-Product Utilization Department, Animal Production Institute, Agriculture Research Center, Nubaria area and Animal Production Department, National Research Center, Dokki, Giza.

Animals and feeding

The present work was lasted 8 weeks and was carried out on 20 sexual mature bucks of NZW rabbits aged about 6 months and weighted 2900 g in average. The animals were divided into 4 equal comparable experimental groups, the first group was fed diet (R1) without addition as control group, while the other three groups were randomly assigned to fed diets added either with 0.1 % *Bacillus s.* (R2); 0.1 % live *Saccharomyces c.* (R3) or mixture of 0.05 % *Bacillus s.* + 0.05 % *Saccharomyces c.* (R4), of Dry matter. Animals were individually housed in wired battery cages supplied with feeders for feeding and nipples for drinking. Fresh tab water was automatically available all the time. All the experimental rabbits were healthy and clinically free from parasites and were kept under the same hygienic conditions. The experimental diets were formed in a pellet form to cover nutrient requirements of the breeding phase of rabbits according to NRC (1994) recommendations. Composition and chemical analysis of the pelleted diet are shown in Table (1). Feed intake were weekly determined for all tested rabbits.

Table (1): Ingredients and chemical analysis of the experimental diets.

Ingredients	%
Ground barley grains	29
Soybean meal (44 %)	20
Yellow corn	8.0
Wheat bran	8.0
Cane-molasses	3.0
Ca.diphosphate	1.0
Sodium chloride	0.5
Minerals and vitamins mixture	0.4
DL-methionine	0.1
Alfalfa hay	30
Chemical analysis, %	
Moisture	10.00
Dry matter composition (DM)	
Organic matter (OM)	93.15
Crude protein (CP)	17.00
Crude fiber (CF)	14.56
Ether extract (EE)	03.44
Nitrogen free extract (NFE)	58.15
Ash	06.85

Semen collected and evaluation:

Semen was collected artificially at the week 5^{th} , 6^{th}, 7^{th} and 8^{th} of experimental period via an artificial vagina. Individual semen samples were evaluated microscopically from each rabbit buck. Semen ejaculate volume (ml), advanced sperm motility (%), sperm cell concentration ($N10^6$/ml), total sperm output ($N10^6$/ejaculate) and sperm abnormalities (%) as well as dead spermatozoa were estimated according to Salisbury *et al.* (1978). Libido (sexual desire) was assessed in terms of reaction time in seconds that was spent from introducing the doe to the buck until the buck start to mount (Daader *et al.* 1999a,b). Testosterone hormone concentration of the rabbit bucks was determined according to the manufacturer information (RIA kits from Immunotech, A Coulter Co., France). In addition, evaluation of seminal initial fructose was carried out immediately after collection according to Mann (1948).

Statistical analysis

Data were subjected to two way analysis of variance according to Steel and Torrie (1980) applying the general linear model procedure of SAS (2001). Significant differences between means were calculated using Duncan's Multiple Range Test (Duncan 1955).

RESULTS AND DISCUSSION

Additives in all experimental diets did not affect daily feed intake of rabbits throughout the experimental period (Fig. 1) from the week 5th until the week 8th.

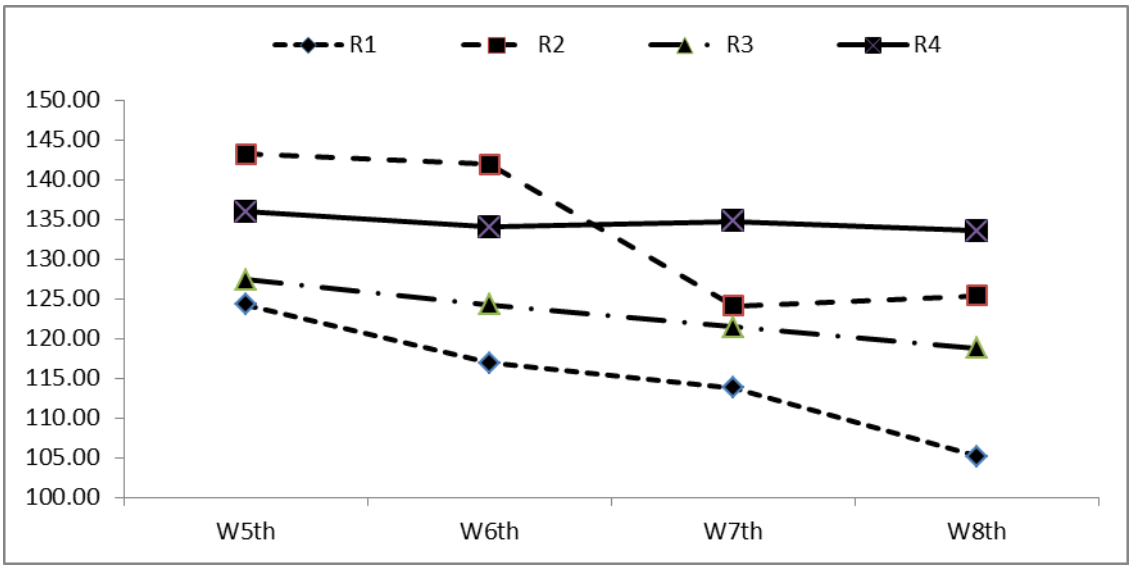

Figure (1): Effect of experimental diets and feeding period on daily feed intake of adult male rabbits.

The effect of the experimental diets on semen characteristics of tested mature male rabbits is presented in Table (2) showed an increases in semen ejaculate volume, total sperm output and sperm cell concentration of rabbits fed supplemented diets (R2, R3 and R4) compared with those fed control diet (R1). It's clear that the best significant (P<0.05) improvement of the previous parameters was obtained by feeding rabbits on diet contained 50 % *Bacillus, s.* + 50 % *Saccharomyces, c.* (R4). These improvements may be due to enhance availability of nutrients facilitated by more efficient nutrients absorption throughout the gastrointestinal tract as a result of probiotics addition (McDaniel and Sefton, 1991), which stimulate the digestion process (El-Badawi, A.Y. *et al.* 2017) that attributed to the highest productive performance and/or contribute to the microbial equilibrium of the gut in order to prevent digestive disorders. This result is in agreement with other researchers who observed remarkably enhancement in total sperm cells and semen ejaculate volume of rabbit bucks, added with antioxidants (Castellini *et al.*, 2003) or probiotics (Ewuola, 2013 and Mymrin *et al.*, 2017). Moreover, total sperm output and sperm cell concentration were significantly (P<0.05) improved by advancing collection period, while semen ejaculate volume was no affected by collection period. Data in the same Table showed that the effect of interaction between the experimental diets and collection period was not significant for all semen characteristics studied.

Table (3) presents the impact of probiotics addition on physical sperm characteristics, data showed that addition of probiotics in diet (R2, R3 and R4) significantly (P<0.05) increased advanced sperm motility and the highest improvement was recorded in R4 compared to R1. These results were in agreement with results obtained by Valcarce *et al* (2017) that the ingestion of probiotics might be recommended to improve sperm motility in human males. There were insignificant (P<0.05) differences among R1, R2 and R3 in sperm abnormality and between R1 and R2 in dead spermatozoa. While the lowest sperm abnormality and dead spermatozoa were recorded in R4.

The effect of collection period regardless experimental diets in the same Table showed that there was a significant (P<0.05) improvement in advanced sperm motility in W7th and W8th compared with W5th and W6th. The lowest significant (P<0.05) sperm abnormality was at W6, W7, and W8 compared with W5th of collection. Additionally, the lowest (P<0.05) percentage of dead spermatozoa was recorded in W7th and W8th compared with W5th and W6th, these results reflected the beneficial effects of probiotics addition with advancing age.

Table (2): Physical semen characteristics of adult rabbit bucks as affected by the experimental diets, collection period and their interaction.

Item		Semen characteristics (mean)		
		Semen ejaculate volume,(ml)	Total sperm output ($Nx10^6$/ml ejaculate)	Sperm cell concentration ($Nx10^6$/ ml)
Effect of experimental diets:				
R1		0.704[b]	216.294[c]	304.063[c]
R2		0.714[b]	233.672[bc]	326.938[bc]
R3		0.728[b]	250.614[b]	344.063[ab]
R4		0.828[a]	293.778[a]	355.313[a]
Effect of collection period:				
W5th		0.704	198.556[c]	282.563[d]
W6th		0.740	234.498[b]	315.625[c]
W7th		0.766	270.578[a]	352.813[b]
W8th		0.764	290.725[a]	379.375[a]
Interaction				
R1	W5th	0.695	191.625	275.000
	W6th	0.665	191.975	287.500
	W7th	0.738	252.125	333.750
	W8th	0.718	229.450	320.000
R2	W5th	0.633	171.663	272.750
	W6th	0.753	229.575	305.000
	W7th	0.713	259.875	370.000
	W8th	0.760	273.575	360.000
R3	W5th	0.715	201.938	285.000
	W6th	0.710	226.205	320.000
	W7th	0.738	260.938	353.750
	W8th	0.750	313.375	417.500
R4	W5th	0.775	229.000	297.500
	W6th	0.833	290.235	350.000
	W7th	0.875	309.375	353.750
	W8th	0.828	346.500	420.000
p- value				
experimental diets		0.001	< 0.0001	0.001
Collection period		0.165	< 0.0001	< 0.0001
Interaction		0.811	0.477	0.203

a,b,c,d Means have different superscripts in the same Column are significantly different at (P<0.05),

The interaction effects appeared on advanced sperm motility and sperm abnormalities but were not clear on percentage of dead spermatozoa. The results show that the advanced sperm motility was significantly (P<0.05) higher for R2, R3 and R4 compared with R1 at W6th, W7th and W8th, respectively. However, sperm abnormalities were significantly (P<0.05) decreased in R4 compared with R1 at W6th and in R2, R3 and R4 compared with R1 at W7th.

These results indicated that rabbits received the probiotics (R2, R3 and R4) had beneficial effects on motility, abnormality and dead spermatozoa.

Results in Table (4) showed the effect of probiotics addition on the male sexual capability measured by lido and level of testosterone hormone as well as initial fructose level. The results of current study

show an improvement in these traits by adding diets with probiotics compared to the control group in spite of the differences were not significant. There were significant (P<0.05) effects of collection period on libido that decreased at W6th, W7th and W8th compared with W5th. However, the interaction effect was not significant.

Table (3): Effect of the experimental diets, collection period and their interaction on some of physical sperm characteristics.

Item		Sperm characteristics (mean)		
		Advanced sperm motility, %	Sperm abnormalities, %	Dead spermatozoa, %
Effect of experimental diets:				
R1		67.875^{c}	25.188^{a}	26.000^{a}
R2		77.500^{b}	23.000^{bc}	26.938^{a}
R3		81.000^{ab}	23.125^{bc}	21.563^{b}
R4		82.375^{a}	21.438^{c}	19.875^{b}
Effect of collection period:				
W5th		71.438^{b}	26.125^{a}	25.938^{a}
W6th		74.938^{b}	22.000^{b}	27.938^{a}
W7th		81.500^{a}	23.313^{b}	19.750^{b}
W8th		80.875^{a}	21.313^{b}	20.750^{b}
Interaction effect:				
R1	W5th	68.750^{ed}	25.250^{bcd}	26.250
	W6th	68.500^{ed}	25.000^{bcd}	28.750
	W7th	67.500^{e}	28.750^{b}	24.750
	W8th	66.750^{e}	21.750^{de}	24.250
R2	W5 th	74.500^{bcde}	26.250^{bcd}	27.750
	W6th	77.750^{bc}	23.250^{cde}	30.250
	W7th	78.250^{bc}	21.500^{de}	23.250
	W8th	79.500^{b}	21.000^{de}	26.500
R3	W5th	72.500^{bcde}	25.500^{bcd}	24.750
	W6th	76.250^{bcd}	21.000^{de}	29.250
	W7th	87.000^{a}	21.500^{de}	14.750
	W8th	88.250^{a}	24.500^{bcd}	17.500
R4	W5th	70.000^{cde}	27.500^{bc}	25.000
	W6th	77.250^{bc}	18.750^{e}	23.500
	W7th	93.250^{a}	21.500^{de}	16.250
	W8th	89.000^{a}	18.000^{e}	14.750
P-value:				
experimental diets		< 0.0001	0.020	0.001
Collection period		< 0.0001	0.001	< 0.0001
Interaction		0.000	0.023	0.449

a,b,c,d,e Means have different superscripts in the same Column are significantly different at (P<0.05),

The enhancement of semen quality in rabbits fed diets supplemented with probiotics especially Bacillus s. and Saccharomyces c. mixture could be associated with higher concentration of testosterone recorded for these groups (Table 4). Particularly, semen ejaculate volume, total sperm output and sperm cell concentration, were improved, in addition to seminal plasma fructose which are testosterone dependent process (Fujihara *et al*., 1983; El-Sherbiny, 1994; Hafez and Hafez, 2000; Hashim *et al*., 2013). In support to this idea, there was an increase in ejaculate volume, sperm motility and sperm concentration when male rabbits treated with testosterone (ElKelawy and Aboulnaga 1995).

Table (4): Libido, testosterone hormone and initial fructose of adult rabbit bucks fed the experimental diets , collection period and their interaction.

Item		Sperm characteristics (mean)		
		Reaction time (Libido), sec.	Testosterone conc. (ng/ml)	Initial fructose conc. (mg/dl)
Effect of experimental diets:				
R1		10.000	2.331	263.588
R2		8.125	2.663	272.528
R3		8.438	2.650	289.813
R4		8.250	2.756	307.420
Effect of collection period:				
$W5^{th}$		10.563^{a}	2.556	296.199
$W6^{th}$		9.625^{ab}	2.675	286.141
$W7^{th}$		7.813^{bc}	2.725	250.965
$W8^{th}$		6.813^{c}	2.444	300.043
Interaction				
R1	$W5^{th}$	9.250	2.725	271.303
	$W6^{th}$	10.500	2.750	263.030
	$W7^{th}$	10.750	2.975	233.903
	$W8^{th}$	9.500	2.575	286.115
R2	$W5^{th}$	10.500	2.500	271.300
	$W6^{th}$	8.500	2.725	271.343
	$W7^{th}$	6.500	2.825	255.168
	$W8^{th}$	7.000	2.550	292.303
R3	$W5^{th}$	11.750	2.525	321.128
	$W6^{th}$	10.000	2.175	289.083
	$W7^{th}$	7.000	2.300	231.163
	$W8^{th}$	5.000	2.325	317.878
R4	$W5^{th}$	10.750	2.475	321.065
	$W6^{th}$	9.500	3.050	321.110
	$W7^{th}$	7.000	2.800	283.628
	$W8^{th}$	5.750	2.325	303.878
P-value:				
experimental diets		0.155	0.329	0.435
Collection period		0.001	0.656	0.308
Interaction		0.264	0.963	0.997

a,b,c, Means have different superscripts in the same Column are significantly different at (P<0.05),

CONCLUSION

In conclusion adding buck's diets with either *Bacillus s.* or *Saccharomyces c.* showed significant (P<0.05) enhancement of reproductive parameters however, the most beneficial effect was observed when both probiotics were combined in diet (50% *Bacillus s.* and 50 % live *Saccharomyces c.*).

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مقاييس جودة السائل المنوى لذكور الارانب النيوزيلاندى المغذاة على علائق مضاف اليها نوعين من البروبيوتيك

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استهدفت هذه الدراسة تقييم استخدام البروبيوتيك لتحسين جودة السائل المنوى لذكور الارانب. تم توزيع 20 ارنب ذكر عشوائيا الي اربع مجاميع للتغذية على اربع علائق تجريبية. وذلك لمدة 8 اسابيع. تم تغذية جميع الارانب على نظام غذائى موحد من العليقة المكعبة حيث كانت عليقة المقارنة R1 بدون اضافات, R2 مضاف اليها Bacillus s. % 0.1 ,R3 مضاف اليها 0.1% c. *saccharomyces*, R4 مضاف اليها Bacillus s. % 0.05 +saccharomyces c. % 0.05. وتم جمع السائل المنوى فى الاسبوع الخامس والسادس والسابع والثامن من التجربة وذلك من كل الارانب المختبرة لتقييم حجم القذفة المنوية, مجموع انتاح الحيوانات المنوية, تركيز الحيوانات المنوية, حركة الحيوانات المنوية, تشوهات الحيوانات المنوية ,الحيوانات المنوية الميتة, هرمون التستوستيرون, تركيز الفركتوز و الرغبة الجنسية.

أوضحت النتائج أن إضافة البروبيوتيك منفردة أو خليط لعلائق ذكور الأرانب حسنت من الكفاءة الانتاجية والفيزيائية لخصائص السائل المنوى ومستوى هرمون التستوستيرون وتركيز الفركتوز ودون التأثير على الرغبة الجنسية وكان التاثير اكثر وضوحا مع الأرانب المغذاة على عليقة مضاف اليها خليط البروبيوتيك 50: 50 % c. *Saccharomysis* + s. *Bacillus*.