Egyptian Journal of Rabbit Science, 26(1): 121 - 134 (2016)

RELATIONSHIP BETWEEN LIPTEN HORMONE CONCENTRATION AND RABBITS REPRODUCTIVITY

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The present work was planned to study the relationship between Lipten hormone concentration and rabbit reproductivity. Two rabbit breeds (Baladi Black as a native breed and New-Zealand White as an exotic breed) were used in the present study. Lipten hormone concentrations were determined in blood, milk and semen of ordinary and low fertile rabbits.

The study included two experiments. Primary experiment was conducted to determine the high and low fertile doe and buck rabbits. The second experiment was planned to measure Lipten hormone concentration in blood serum of high and low fertile doe and buck rabbits. Lipten hormone levels were also measured in milk and semen of lactating doe and buck rabbits, respectively.

Sixty sexually mature rabbits (30 in each of low-fertile and high-fertile) of twelve months of age (10 does & 5 bucks within each breed of Baladi Black "BB" and New-Zealand White "NZW") were used in this study.

Results Obtained showed that,blood swrum Leptin hormone concentration in of high fertile doe and buck rabbits were significantly (P≤0.5 or 0.1) higher than those in low fertile ones, in both two breeds (BB and NZW) rabbits. In each status studied, high and low fertile, and doe and buck rabbits, BB rabbits recorded leptin hormone levels insignificantly higher than those obtained by NZW rabbits.

High fertile BB and NZW lactating rabbit does produced milk with Leptin hormone concentration significantly(P≤0.5 or 0.1)higher than recorded by low fertile rabbits, during nursing period up to 5 weeks. Semen ejaculated by high fertile BB and NZW rabbit bucks

contained Leptin hormone levels more significantlyhigher (P≤0.5 or 0.1) than those of low fertile bucks.

Coclusively, *it could be concluded that, there was strongly correlation between Leptin hormone concentration in blood, semen and milk and rabbit reproductivity. BB rabbits recorded Leptin hormone levels insignificantly higher than those of NZW rabbits, so BB rabbits may be characterized by reproductive capability more than NZW rabbits.*

Key words: Rabbit; Leptin; fertility; blood; semen; milk.

Developing countries like Egypt are often suffered from protein deficiency. This is due to two main factors. The first is the small number of existing farm animals as compared to rapid growth of human population, and the second is the low productive and reproductive capabilities of these animals (Daader and Seleem, 1999 and Rowida *et al.*, 2010).

The increase in animal protein production may come from short-life cycle animals kept by the small holder farmers such as rabbits (Galal and Khalil, 1994). Rabbit industry is the hope can display an essential role in solving a part of meat shortage and minimizing the gap between requirement and available of the animal protein for human consumption (Zaki *et al.*, 2000; Seleem *et al.*, 2006 and 2007 and Rowida and Seleem, 2007). So, rabbits had gradually increased attention in the last few months for meat production.

Zhang *et al.* (1994) demonstrated that, Lipten has additional physiological activities, including activation of the sympathetic nervous system, regulation of reproductive function, and activation of the immune system. In human; Houseknecht *et al*. (1997) reported that, Lipten concentrations in whole and skim milk were correlated with maternal plasma Lipten concentrations. In sheep circulating lipten levels increased from early tomid pregnancy and remained elevated until late pregnancy

Therefore, the aim of the present work was to evaluate the relationship between Lipten hormone levels and rabbit reproductivity, using Baladi Black as a native breed and New-Zealand White as an exotic breed.

MATERIALS AND METHODS

The present study was conducted in the Department of Poultry Production, Faculty of Agriculture, Ain Sham University, Qalubia, Egypt, in

partnership with Department of Rabbit, turkey and Waterfowl Breeding Research, Animal Production Research Institute, AgriculturalResearch Center, Dokki, Giza, Egypt. The experimental work was carried out in an Industrial Rabbitry of International IBEX company, near Sakara city, Giza Province, Egypt. The study lasted three months, during the period from January till March.

Experimental animals

Sixty sexually mature rabbit 30 for each low fertile and high (high _fertile) of twelve month of age (10 does 5 bucks within each breed were used in this study.

Animals were healthy and clinically free of external and internal parasites. Body weight of each animal was recorded twice monthly to make sure that, the animals were healthy.Rabbits were fed *ad libitum* a commercial diet according to NRC (1994). Ingredients and chemical composition of the pelleted rations are shown in Table 1. Rabbits were raised in semi-closed Rabbitry with wire-netted windows on their sides for providing natural ventilation. The windows were oriented with an elevation of 2 meters from the floor. The floor was made from concrete and have moderately slope (from the middle to both sides) to facilitate water drainage towards a large gutter outside the Rabbitry. During winter and windy days, windows were covered with canvas cloth for protecting rabbits from severe atmosphere especially at night. Rabbits were housed separately in individual wired-cages and arranged in double-tier batteries along the Rabbitry. Cage of each doe (maternity cage) was provided with a metal nest box for kindling and nursing her young, during the suckling period. All cages were equipped with feeders (made of galvanized steel sheets) and have nipples (automatic drinkers). The system provided animals with fresh water and diet which were offered *ad libitum* all over the experimental period. During the experimental work, the Rabbitry was lit artificially 16 hours daily. Rabbits were kept under similar management system.

The experimental work:

Experiment 1:

Primary experiment was conducted to determine the high and low fertile of both doe and buck rabbits. The experiment was designed to separate low-fertile does from high-fertile ones of BB and NZW rabbits. The separation depended on the records in Rabbitry represented in rates of each of

Table 1. The ingredients and chemical composition of thepellet expermental ration

Ingredients	**(%)**	**Vitamins & Minerals premix per Kilogram.**	
Clover hay	40.50	Vit.A (IU)	10000
Wheat bran	25.00	Vit.D3 (IU)	9000
Yellow corn	14.00	Vit.E (IU)	10000
Soybean meal (44%)	11.00	Vit.K (IU)	3
Molasses	3.00	Vit.B1 (IU)	2
Vinass	3.00	Vit.B2 (IU)	6
Bone meal	1.75	Vit.B6 (IU)	2
Lime stone	0.70	Biotin (mg)	0.2
Sodium chloride	0.55	Choline (mg)	1200
Vitamins & Mineral Premix	0.35	Niacine (mg)	40
DL-Methionine	0.15	Zn. (mg)	60
Total	**100**	Cu. (mg)	0.1
Calculated chemical composition **		Mn. (mg)	85
Crudeprotein (CP)%	18.00	Fe. (mg)	75
Ether extract (EE)%	3.00	Folic acid (mg)	5.0
Crude fiber (CF)%	14.00	Pantothenic acid (mg)	20
Digestible energy (Kcal/Kg)	2720.00		

** Calculated according to **NRC (1994)** for rabbits.

abortion, conception and kindling and values of each of litter size and weight at birth and at weaning, in addition to bunny weight at birth and at weaning. The pattern used to determine the fertility traits of rabbit does according to Rabbitry records are shown in Table 2. BB and NZW rabbit bucks characterized by low-fertilizing ability were also, separated from those with high-fertilizing ability using libido and physical semen characterized represented in (semen-ejaculate volume, mass and advanced sperm motility, percentages of dead and abnormal spermatozoa and acrosomal damages and sperm-cell concentration per ml and per ejaculate), in addition according to mating activity, scrotal circumference and testicular index. The pattern used to evaluate fertilizing ability of rabbit bucks as recorded by physical semen characteristics are shown in Table 3.

Sixty sexually mature rabbits (30 in each of low-fertile and high-fertile) of twelve months of age (10 does & 5 bucks of each breed of Baladi Black "BB" and New-Zealand White "NZW") were used in this study.

Table 2. The pattern used to determine the fertility traits of rabbit does according to Rabbitry records.

Items	Fertility traits	
	Low-fertile	**Ordinary-fertile**
Abortion rate (%)	≥ 03.00	< 03.00
Conception rate (%)	≤ 60.00	> 60.00
Kindling rate (%)	≤ 57.00	> 57.00
Litter size at birth (No.)	≤ 04.75	> 04.75
Litter weight at birth (g)	≤ 200.0	> 200.0
Bunny weight at birth (g)	≤ 40.00	> 40.00
Litter size at weaning (No.)	≤ 04.00	> 04.00
Litter weight at weaning (g)	≤ 2250	> 2250
Bunny weight at weaning (g)	≤ 0550	> 0550

Table 3. The pattern used to evaluate fertilizing ability of rabbit bucks.

Items	Fertilizing ability	
	Low-fertile	**Ordinary-fertile**
Libido (Sec.)	≥ 40.00	< 40.00
Mass sperm motility (Score)	≤ 02.50	> 02.50
Advanced sperm motility (%)	≤ 50.00	> 50.00
Dead spermatozoa (%)	≥ 35.00	< 35.00
Sperm abnormalities (%)	≥ 30.00	< 30.00
Acrosomal damages (%)	≥ 25.00	< 25.00
Semen ejaculate volume (ml)	≤ 00.40	> 00.40
Sperm-cell concentration (N X 106/ ml)	≤ 450.0	> 450.0
Total-sperm output (N X 106/ ejaculate)	≤ 180.0	> 180.0
Mating activity (no. of mating/ 20 minutes)	≤ 02.50	> 02.50
Scrotal circumference (Cm)	≤ 07.00	> 07.00
Testicular index (Cm3)	≤ 06.50	> 06.50

Experiment 2:

It was planned to measure Lipten hormone concentration in blood serum of doe and buck of BB and NZW rabbits. *Lipten* hormone levels in milk, during lactating period of BB and NZW rabbit does were recorded weekly up to the 5th week, as well as, *Lipten* hormone levels were estimated in 1st and 2nd semen ejaculated by BB and NZW rabbit bucks. *Lipten* hormone levels were measured in ordinary and low-fertile does and in bucks of high and low-

fertilizing ability of two rabbit breeds used in the study.Five bucks and ten does within both high and low-fertile of each rabbit breed used in the study were conducted to determine *Lipten* hormone levels in blood, semen or milk.

Measurement of Leptin hormone concentration

Leptin hormone concentration in blood serum and milk and seminal plasma was done using DRG® *Leptin* (Sandwich) ELISA (EIA-2395). The DRG® *Leptin* ELISA commercial kit is a solid phase enzyme-linked immunosorbent assay (ELISA) accordin to Considine and Sinha (1996) and Guillaume and Bjorntorp (1996).

Blood collection

Blood samples were collected from the marginal ear vein of 3 does and 3 bucks of eachexpermental group after shaving and cleaning with alcohol. A spring lancet made of steel sheets puncture was used to get an adequate but not profuse flow of blood. Blood samples were collected at the morning between 8.00 to 10.00 a.m. as stated by Thompson and Proctor (1984).

Blood samples were collected into dry clean heparinzed centrifuge tubes. With heparin film to prevent the blood coaggulant . Blood serum was separated by centrifugation at 3000 r.p.m. for 20 minutes and kept in a deep freezer at -20C until analysis.

Semen collection

Semen was collected using artificial vagina between 08.00 to 10.00 a.m. As described by Waston

Semen evaluation

Libido and physical semen characteristics represented in values of each of semen-ejaculate volume; mass and advanced sperm motility; percentages of sperm abnormalities; dead spermatozoa and acrosomal damages and sperm-cell concentration per ml and per ejaculate were evaluated. Physical semen characteristics were evaluated according to Campbell *et. al.* (1956);Hackett and Macpherson (1965); Watson (1975); Salisbury*et. al* (1978) and Seleem *et al.* (2009).

Mating activity of rabbit buck was done as studied by Badawi *et. al.* (2010). Scrotal circumference wasmeasured by measuring the circumference of scrotum of each testis of each BB and NZW rabbit bucks as the method

described by Mickelsen *et. al.* (1982).Testicular index (length xwidth x depth) was calculated in cubic centimeters as recorded by El-Kholy *et. al.* (2012).

Statistical analysis

Data were statistically analyzed using Least Squares Analysis of Variance according to Snedecor and Cochran (1967). Percentage values were transformed to arcsin values before being statistically analyzed. Duncan's Multiple Range Test (Duncan, 1955) was used to compare the differences between significant means. Conception and kindling rates were analyzed using Contingency tables according to Everitt (1977).

RESULTS AND DISCUSSIONS

Leptin Hormone levels in blood

Data presented in Tables (4 and 5) showed that, Leptin hormone concentration in blood serum of ordinary fertile doe and buck rabbits were significantly ($P \leq 0.5$ or 0.1) higher than those recorded in low fertile ones. This trend was recorded in both two studied breeds (BB and NZW) rabbits., BB rabbits recorded Leptin hormone levels insignificantly higher than those obtained by NZW rabbits. For both high and low fertile or both does and bucks.

Leptin Hormone levels in milk

Table 6 clearly showed that, ordinary fertile BB and NZW lactating rabbit does produced milk with Leptin hormone concentrations significantly highly ($P \leq 0.01$)those recorded by low fertile rabbits, during nursing period from kindling up to 5 weeks. BB rabbit does show values of leptin hormone concentrations insignificantly superior than those recorded by NZW ones.

Leptin Hormone levels in semen

Data presented in Table 7 showed that, semen ejaculated by high fertile BB and NZW rabbit bucks included Leptin hormone concentrations highly significant ($P \leq 0.01$) higher than those of low fertile bucks. BB rabbit bucks and second ejaculates characterized by Leptin hormone levels insignificantly higher than those obtained by NZW rabbit bucks and first ejaculates, respectively.

Table 4. Leptin hormone levels in blood (ng/ dl) of low and high fertile rabbit does (Means ± SE).

Breeds	Fertility traits		Means ± SE
	Low-fertile	high-fertile	
BB	2.47±0.42	3.84±0.51	3.16±0.44
NZW	2.36±0.37	3.61±0.54	2.99±0.49
Means ± SE	2.42±0.63 b	3.73±0.53 a	3.07±0.57

Means bearing different letter superscripts within the same row are significantly ($P \leq 0.01$) differ

Table 5. Leptin hormone levels in blood (ng/ dl) of low and high fertile rabbit bucks (Means ± SE).

Breeds	Fertility traits		Means ± SE
	Low fertile	high fertile	
BB	2.34±0.37	3.62±0.52	2.98±0.41
NZW	2.24±0.33	3.43±0.54	2.84±0.40
Means ± SE	2.29±0.34 b	3.53±0.50 a	2.91±0.46

Means bearing different letter superscripts within the same row are significantly ($P \leq 0.01$) differ.

Table 6. Leptin hormone concentration in milk (ng/ dl) of low and high fertile rabbit does (Means ± SE).

Milk period	Breeds	Fertility traits		Means ± SE
		Low-fertile	highcc-fertile	
1st week	**BB**	1.93±0.27	3.36±0.59	2.65±0.36
	NZW	1.84±0.19	3.28±0.62	2.56±0.21
Means ± SE		1.89±0.26 b	3.32±0.54 a	2.61±0.33
2nd week	**BB**	2.51±0.32	3.69±0.70	3.10±0.42
	NZW	2.32±0.34	3.64±0.63	2.98±0.52
Means ± SE		2.42±0.33 b	3.67±0.68 a	3.04±0.51
3rd week	**BB**	2.64±0.44	3.99±0.58	3.32±0.54
	NZW	2.41±0.51	3.92±0.48	3.17±0.47
Means ± SE		2.53±0.46 b	3.96±0.56 a	3.24±0.50
4th week	**BB**	2.11±0.38	3.47±0.47	2.79±0.40
	NZW	2.01±0.30	3.18±0.40	2.60±0.34
Means ± SE		2.06±0.31 b	3.33±0.46 a	2.70±0.33
5th week	**BB**	1.86±0.25	3.14±0.59	2.50±0.29
	NZW	1.79±0.18	2.95±0.46	2.37±0.22
Means ± SE		1.83±0.22 b	3.05±0.51 a	2.44±0.33
Overall means ± SE		2.15±0.24 b	3.47±0.46 a	2.81±0.27

Means bearing different letter superscripts within the same row are significantly ($P \leq 0.05$ or 0.01) differ.

Table 7. Leptin hormone concentration in semen (ng/ dl) low and high fertile rabbit bucks (Means ± SE).

Semen ejaculated	Breeds	Fertility traits		Means ± SE
		Low-fertile	Ordinary-fertile	
1st ejaculate	BB	2.07±0.28	3.21±0.42	2.64±o.33
	NZW	2.02±0.31	3.09±0.33	2.56±0.30
Means ± SE		2.05±0.28 b	3.15±0.36 a	2.60±0.31
2nd ejaculate	BB	2.22±0.19	3.48±0.44	2.85±0.26
	NZW	2.16±0.24	3.26±0.29	2.71±0.22
Means ± SE		2.19±0.20 b	3.37±0.33 a	2.78±0.24
Overall means ± SE		2.12±0.17 b	3.26±0.33 a	2.69±0.21

Means bearing different letter superscripts within the same row are significantly ($P \leq 0.05$ or 0.01) differ.

Zhang *et al*. (1994) identified the mutated protein, Lipten, in mice, which is assumed to be the circulating satiety signal. Lipten has additional physiological activities, including activation of the sympathetic nervous system, regulation of reproductive function, and activation of the immune system (Zhang *et al.*, 1994). In human; reported that, Lipten concentrations in whole and skim milk were correlated with maternal plasma lipten concentrations. From early to mid-pregnancy, circulating Lipten levels increased and remained elevated until late pregnancy in sheep. In mares, Romagnoli*et al*. (2007) found that, the highest cholesterol lipten level recorded during the week of parturition compared with two weeks before parturition. On the other hand, in sows Huszeniczaet *al.* (2002) and Smith and Grove (2002) reviewed that, negative energy balance during lactation is reflected by decreases in serum Lipten and thyroid hormone levels. At this time, it is unclear and poorly documented, what mechanisms may be responsible for the suppression of thyroid hormones and whether there is a link to the suppression of Lipten.

New Zealand White rabbits, as well known in Egypt as a meat purpose breed that intensively spread all over the country Ayyat and Marai (1998) have indicated that, Baladi Black rabbits as a local meat type breed are widely and more adapted with Egyptian environmental conditions.

Khalil *et al*. (1988) and Hilmy (1991) scored that, local Baladi Black rabbits, which assumed to be adapted to the Egyptian conditions have

different genetic basis among foreign breeds. On the other hand, found NZW rabbit breed characterized by productive and reproductive superior than Baladi Black ones. Direct genetic effects of exotic breeds compared to Baladi ones are favorable in letters at birth and during the first 21 days of sucking but not at weaning (Afifi and Khalil, 1991). New Zealand White bucks generally produce litters with larger size and heavier weight along with heavier mean bunny weight at birth and at 21 days of age than do the Baladi bucks (Youssef, 1992).

The study current concluded that, there was strongly correlation between Leptin hormone concentration in each of (blood, semen and milk) and rabbit reproductivity. BB rabbits recorded Leptin hormone levels insignificantly higher than those of NZW rabbits, so BB rabbits may be characterized by reproductive capability more than NZW rabbits, under the environmental conditions of conducting that experiment.

Coclusively, it could be concluded that, there was strongly correlation between Leptin hormone concentration in blood, semen and milk and rabbit reproductivity. BB rabbits recorded Leptin hormone levels insignificantly higher than those of NZW rabbits, so BB rabbits may be characterized by reproductive capability more than NZW rabbits.

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العلاقة بين تركيز هرمون اللبتين والتناسل فى الأرانب

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صمم هذا البحث لدراسة العلاقة بين تركيز هرمون اللبتين والتناسل فى الأرانب. وقد أستخدم سلالتين من الأرانب (البلدى الأسود كسلالة محلية، والنيوزيلندى الأبيض كسلالة أجنبية ومتأقلمة مع الظروف المصرية)، وتم قياس تركيزات هرمون اللبتين فى كل من الدم واللبن والسائل المنوى لكل من الأرانب منخفضة وعالية الخصوبة.

وقد إشتملت الدراسة على تجربتين أساسيتين، أجريت التجربة الأولى لتحديد ذكور وإناث الأرانب منخفضة وعالية الخصوبة. أما التجربة الثانية فقد صممت لقياس تركيزات هرمون اللبتين المختلفة فى دم ذكور وإناث الأرانب منخفضة وعالية الخصوبة وكذلك تم قياس مستويات هرمون اللبتين فى لبن الأمهات المرضعات وسائل منوى ذكور الأرانب منخفضة وعالية الخصوبة.

أستخدم فى هذه الدراسة عدد ٦٠ أرنب ناضج جنسياً (٣٠ أرنب فى كل من منخفض وعالى الخصوبة)، عمر ١٢ شهراً، (١٠ إناث و٥ ذكور فى كل سلالة من البلدى الأسود والنيوزيلندى الأبيض).

أوضحت النتائج أن: تركيزات هرمون اللبتين فى دم ذكور وإناث الأرانب عالية الخصوبة كان أعلى معنوياً (عند مستوى ٥ أو ١%) عن مثيلاتها فى الأرانب منخفضة الخصوبة، فى كلا السلالتين المستخدمتين فى الدراسة (البلدى الأسود والنيوزيلندى الأبيض). وقد تبين أن إناث وذكور أرانب البلدى الأسود سجلت مستويات هرمون اللبتين أعلى غير معنوياً عن تلك التى سجلتها أرانب النيوزيلندى الأبيض، فى كلا الحالتين تحت الدراسة (منخفضة وعالية الخصوبة).

أمهات أرانب البلدى الأسود والنيوزيلندى الأبيض عالية الخصوبة والمرضعات أنتجت لبن بمستويات هرمون اللبتين أعلى معنوياً (عند مستوى ٥ أو ١%) عن تلك المنتجة مع لبن الأمهات منخفضة الخصوبة، خلال فترة الرضاعة حتى خمسة أسابيع. سجلت إناث أرانب البلدى الأسود قيم لمستويات هرمون اللبتين فى اللبن أعلى غير معنوى عن تلك المسجلة فى لبن إناث النيوزيلندى البيضاء. مستويات هرمون اللبتين المسجلة كانت مرتبة ترتيباً تنازلياً غير معنوى عند الإسبوع الثالث، الثانى، الرابع، الأول ثم الخامس، على الترتيب، خلال فترة الرضاعة. كان السائل المنوى المقذوف من ذكور أرانب البلدى الأسود والنيوزيلندى الأبيض عالية الخصوبة يحتوى على مستويات لهرمون اللبتين أعلى معنوياً (عند مستوى ٥ أو ١%) عن تلك الموجودة فى سائل منوى الأرانب منخفضة الخصوبة. كانت مستويات هرمون اللبتين التى سجلتها ذكور أرانب البلدى الأسود، وفى القذفة الثانية من السائل المنوى أفضل غير معنوياً عن تلك المسجلة فى سائل منوى أرانب النيوزيلندى الأبيض، وفى القذفة المنوية الأولى، على الترتيب.

التوصية: ويمكن أن نستخلص من الدراسة أن كان هناك أرتباط قوى بين مستويات هرمون اللبتين فى كل من الدم، والسائل المنوى ، واللبن وبين التناسل فى الأرانب. أرانب البلدى الأسود سجلت مستويات لهرمون اللبتين أعلى غير معنوياً عن تلك التى سجلت فى الأرانب النيوزيلندى البيضاء، ولذلك فإن أرانب البلدى الأسود ربما تمتاز بمقدرة تناسلية أعلى عن النيوزيلندى البيضاء.

Egyptian Journal of Rabbit Science, 26(1): 121 - 134 (2016)