

EFFECT OF ADMINISTRATION OF L-TYROSINE AND L-CARNITINE ON ONSET OF PUBERTY IN RABBITS

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SUMMARY

The present study was conducted on 72 New Zealand white rabbits assigned into two age groups (two and three months): 36 rabbits for each group of the same age (18 males and 18 females). For treatment, 24 rabbits (males and females) were given orally a single dose of L-tyrosine, 100 mg/kg body weight; the other 24 rabbits (males and females) were given a daily oral dose of 1 gm L-carnitine/kg. The other 24 rabbits were left as control. All rabbits were kept under complete observation during the experimental period and data obtained were statistically analyzed. There was a steady increase in the body weight with advancement of age; it seemed to be similar for males and females. The treated group revealed a significant higher body weight ($P<0.05$) when compared to control and treated females, exhibited higher body

gain than males. In the treated group, the testicular descent in males and the first onset of male acceptance by females occurred much earlier during the second month of age compared to control. Successful mounting and ejaculation of semen containing spermatozoa for the first time was recorded in the treated bucks at the beginning of the third month of age, much earlier than in control. Testes and ovaries size increased significantly with advancement of age. L-tyrosine and L-carnitine treated groups revealed significant increase in the testicular and ovarian size compared to those in the control group ($P<0.01$). It could be concluded that L-tyrosine and L-carnitine, both act as growth promoting factors. They could be used in rabbits for enhancement of puberty in both males and females.

INTRODUCTION

For several reasons, rabbits are unique among small animals for food and commerce. They produce highly nutritious, low-fat and low-cholesterol meat which is rich in proteins, certain vitamins and minerals. Being herbivores, they do not compete with humans for their food and are easily adaptable to different environments, and investment and labor costs are low. They are easy to transport and market for food, fur and skin. In addition they are highly productive, characterized by long breeding season, which may extend from October to May, associated with a large number of litters with short gestation and lactation periods (Daader et.al., 2002). Puberty of the male rabbit has been defined as the first time when the male becomes capable for producing spermatozoa in the ejaculate (Sand, 1979). It has also been defined as the stage when the endocrine function of the testes first becomes clearly evident (Donovan and Van der werfften Bosch, 1965). Puberty occurs a month or even more before rabbits attain sexual maturity, and there is an age difference between the different breeds and strains of rabbits (Sand, 1979). The small white Polish bucks reach puberty at four months of age, whereas New Zealand white and Californian bucks reach puberty at five months of age (Peter, et.al., 1979).

Based on the use of some organic substances used to improve the reproductive efficiency of animals through enhancing the release of sex hormones,

the present study aimed at studying two of these substances: L-Tyrosine and L-Carnitine. L-Tyrosine is a semi essential amino acid involved in the formation of catecholamine from adrenal gland and thyroxin in the thyroid gland (Harmmerl and R, sse, 1987). L-Carnitine is a dipeptid amino acid. Sex hormones are steroid hormones, and all steroid hormones are ultimately made from a single precursor, cholesterol, which in turn is made from acetyl-CoA that is formed through the carnitine transport (Lehninger, 1982).

L-Carnitine performs the following tasks in relation to spermatogenesis and function of mammalian spermatozoa; 1) Synthesis and protection of cell membranes (Uhlenbruck,1996), 2) Storage of activated acetic acid in the form of acetylcarnitine, which is an energy-rich compound that serves as the primary energy source for sperm motility after insemination (Jeulin et.al.,1988). Trials have indicated that, L-carnitine has beneficial effects in both productive and reproductive performance of monogastric species including sows (Harmeyers, 1993 and Freemaut et. al., 1993), mares (Iben and Leibertseder, 1994) and poultry (Leibertseder and Iben, 1994). In contrast to monogastric species the energy supply of ruminants is based almost entirely on oxidation of short and long chain fatty acids rather than carbohydrates. L-carnitine is therefore, even more important for ruminant than monogastric species, Noseir and Amrawi (2000) found an improvement effect of L-carnitine supplementation on fertility

of normal and sub fertile rams.

L-tyrosine has been found to act as a growth promoting factor and hormonal inducing factor in rabbits (Abo-Elroos, 1992), rats (Scheuermann, 1984), dogs (Aumann, 1987), pigs (Gindele and Koppen, 1988), and buffaloes (Amrawi et.al., 1992; Amrawi, 1997). It is of interest to study the changes in activities of gonads by administration of those amino acids, however, it is difficult to confirm these changes in mature animals in which gonads are normally functioning, and therefore selecting animal just before reaching puberty is helpful to study the effect of these amino acids on gonads. The present study was designed to study the effect of administration of either L-tyrosine or L-carnitine on the onset of puberty in rabbits.

MATERIALS AND METHODS

Animals: Seventy two (36 males and 36 females) immature New Zealand white rabbits aged 2-3 months and bred at the Faculty of Veterinary Medicine, Alexandria University during the period from January to April were used. Rabbits were housed individually inside steel cages, with free access to clean water via automatic water troughs and feeding on a constant plan of nutrition throughout the period of the experiment. The daily minimum and maximum indoor temperatures ranged between 14 and 20°C. A lightening schedule of 12h of light was used. Each animal was allowed a daily non-treated ration composed of

about 200g concentrates (including 27% barley, 25% wheat bran, 20% ground corn, 15% ground horse beans, 8% soybean meal, 1.5% bone meal, 3% minerals and vitamins, and 0.5% common salt), in addition to barseem hay ad libitum. Rabbits were given a period of adjustment for at least two weeks before reaching the starting age of experiment (2 and 3 months), to ensure a good health condition and to avoid stress during transportation, through the daily observation of any clinical signs indicating infection or parasitic infestation.

Experiment: Rabbits were assigned according to the age at the beginning of the experiment into two equal groups; A and B aged 2 and 3 months, respectively. Each group contained, 18 males and 18 females;

Group A (2 months old): both sexes were divided into 3 equal subgroups: the first of which (6 males and 6 females) was treated by administering 100 mg L-tyrosine/kg body weight (Theriogon-ADWIA), in a single oral dose by direct dilution into 3 ml sterile water. Each animal received a separate dose orally. The second subgroup (6 males and 6 females) was treated by oral administration of 1gm L-carnitine/kg body weight (Carniking 50%-Pharopharma Egypt) by direct dilution into 5 ml sterile water. Each animal received the dose as in the first subgroup and the treatment was given daily for two weeks. The last subgroup (6 males and 6 females) was kept as control.

Group B (3 months old): were divided into three subgroups and were treated similarly as in group A.

Clinical observation started on day zero at the beginning of the study for one month period. Both the control and treated groups were kept under continued observation to estimate the following criteria:

- 1- body temperature ($^{\circ}\text{C}$); was measured by a digital thermometer, twice daily early in the morning and late evening for a week. Daily average was taken and recorded.
- 2- body weight (kg) was estimated weekly, the body weight was measured by digital scale and recorded for each individual to be statistically analyzed to determine the level of body gain and compare among treatment and control groups.
- 3- Sexual development (The descent of the testis into the scrotum); testicular descent was observed daily for Group A, to determine the degree of male maturity and effect of treatments.
- 4- Sexual interest was detected by checking the first male acceptance by the female. This was done every two days for the 2 months old does, by introducing a female to a mature male cage and recording the results.

The size of testicles and ovaries was recorded according to Ismail (1979), to show the differences and compare between treatment and control

groups within the same age groups. Numbers of kids were recorded among experimental and treatment groups.

Statistical analysis was performed using the least square difference (LSD) between groups (Nouris, 1986).

RESULTS

Clinical observations:

1- Body temperature: There was a slight tendency for elevation in body temperature of L-carnitine and L-tyrosine than control in both groups (A and B), although the statistical analysis (Table, 1) revealed that differences between treated and control groups were not significant.

2- Body weight: the effects of sex, age and treatment (L-tyrosine and L-carnitine) on body weight and net body gain, are presented in Table, 2. A significant increase in net weight gain was observed in treated groups compared to control.

3- Sexual development: Daily observation of rabbits throughout the experimental period showed that the testicular descent was completed in the male and the first onset of male acceptance was shown by the female rabbits at two months of age. Results indicated that when L-tyrosine was used, the testicular descent occurred at 11-12th day from the second month

of age, while in L-carnitine treated group the testicular descent occurred at 14-15th day after the second month of age. This was remarkably earlier than in the control (27-28th day). When females were checked daily for standing to be mated by males as a sign of sexual development, it was noticed that the first time for male acceptance by females was much earlier in tyrosine-treated group (10-11th day) and in carnitine group (13-15th day) beginning from the second month of age compared to control (21-23rd day).

4- Sexual interest was recorded in the tyrosine-treated bucks at the beginning of the third month of age (90-97 days), while in carnitine

group at 85-95 days, whereas control bucks displayed sexual interest at approximately the middle of the fourth month for the four months old rabbits (131-133 days).

Changes in the testicular and ovarian size of experimental groups are presented in Table, 3. There was a significant statistical difference ($P < 0.01$) in ovarian size between tyrosine treated group and both control and carnitine groups.

Number of kids in treatment groups (Table, 4) in both experimental groups (A&B) were significantly ($P < 0.05$) higher than control.

Table (1):- Effect of age and treatments (L-tyrosine and L-carnitine) on body temperature of rabbits ($^{\circ}\text{C}$)

	Group A (2 Months old)	Group B (3 Months old)	Overall mean
L-Tyrosine	38.45 \pm 0.34 ^a	38.59 \pm 0.15 ^a	38.51 \pm 0.22 ^a
L-carnitine	38.55 \pm 0.08 ^a	38.73 \pm 0.11 ^a	38.61 \pm 0.10 ^a
Control	38.31 \pm 0.24 ^a	38.48 \pm 0.17 ^a	38.41 \pm 0.19 ^a

Means \pm SE. Means with different alphabetical superscripts (a,b,..) in the same column are significantly different ($P < 0.05$).

Table (2):- Effect of age, sex and treatment (L-tyrosine and L-carnitine) on body weight and net body gain of rabbits.

Group (A)	Starting b.w.(kg)	After 1 Month(kg)	Net Weight gain(kg)
Male			
L-tyrosine	2.16±0.25	2.89±0.16	0.73±0.29 ^a
L-carnitine	2.00±0.31	2.71±0.17	0.71±0.31 ^a
Control	2.43±0.06	2.85±0.15	0.42±0.21 ^b
Female			
L-tyrosine	2.05±0.42	3.12±0.17	1.07±0.16 ^a
L-carnitine	2.00±0.44	3.06±0.55	1.06±0.27 ^a
Control	2.33±0.11	3.15±0.38	0.82±0.23 ^b
Group (B)	Starting b.w. (kg)	After 1 month(kg)	Net Weight gain(kg)
Male			
L-tyrosine	2.80±0.08	3.10±0.12	0.30±0.10 ^a
L-carnitine	2.86±0.12	3.12±0.17	0.26±0.08 ^a
Control	2.80±0.04	2.99±0.04	0.19±0.09 ^b
Female			
L-tyrosine	3.11±0.56	3.43±0.77	0.32±0.06 ^a
L-carnitine	3.17±0.18	3.49±0.39	0.32±0.08 ^a
Control	3.20±0.40	3.52±0.61	0.32±0.04 ^a

Means ± SE. Means with different alphabetical superscripts (a,b,..) in the same *column* are significantly different (P<0.05).

b.w. = body weight (kg).

Table (3):- Interaction of age and treatment (L-tyrosine and L-carnitine) on Testicular and ovarian size of rabbits (cm³).

	Group A (2 Months old)	Group B (3 Months old)
Testicular size		
L-carnitine	2.59±0.05 ^a	2.85±0.20 ^a
L-tyrosine	2.61±0.04 ^a	3.06±0.10 ^a
Control	1.59±0.10 ^b	1.82±0.06 ^b
Ovarian size		
L-carnitine	1.03±0.08 ^a	1.09±0.03 ^a
L-tyrosine	1.24±0.07 ^a	1.41±0.07 ^b
Control	0.46±0.04 ^b	1.06±0.04 ^a

Means ± SE. Means with different alphabetical superscripts (a,b,..) in the same *column* are significantly different at P<0.01.

Table (4):- Interaction of age and treatments on the number of kids.

	Group (A)	Group (B)	Overall mean
L-carnitine	6.5±1.2 ^a	6.7±0.9 ^a	6.6±1.0 ^a
L-tyrosine	6.7±1.5 ^a	6.5±1.2 ^a	6.6±1.3 ^a
Control	4.7±0.9 ^b	5.5±1.0 ^b	5.1±0.9 ^b

Means ± SE. Means with different alphabetical superscripts (a,b,..) in the same *column* are significantly different at P<0.05.

DISCUSSION:

It is well known that a phenomenon of the body metabolism interpreting a change in the physiological activity of an individual is to find a corresponding change in the body temperature. Thus, increasing the body metabolism seems to have a positive relationship with body temperature. Although, in the present study, differences in the body temperature recorded (Table, 1) among experimental and treatment groups were not significant, but higher body temperature appeared with the rabbit approaching age of puberty was observed in both treatment groups.

In the meantime advancement of age of rabbits under the present experimental condition was noticed to have a significant positive relationship to the body weight. The net gain in the body weight appeared more prominent in rabbits of two and three months (Table, 2), a finding which also supported the previous suggestion that puberty in rabbits starts with the third month of age (Lebas et al., 1986), contrary to that mentioned in some earlier studies (Arrington and Kelly, 1976; Peter et al., 1979; Sand, 1979). Throughout the different ages when tyrosine was administered, the body weight of the treated rabbits revealed a highly significant increase when compared to that of control. It could be speculated that tyrosine acts as a growth promoting factor. This is in accordance with the earlier reports showing that in-

creasing the circulating tyrosine activates the hypothalamic catecholamine, dopamine, which in turn activates the release of growth hormone (Miller, 1973), in addition to its importance in formation of thyroxin and protein synthesis (Harper et al., 1980).

Throughout the age of two months group when carnitine was supplemented, it produced positive and significant effect on growth rate compared to control. This finding comes in accordance with studies conducted on sows (Musser et al., 1999).

From the present study, it has been shown that testicular descent into the scrotum takes place much earlier during the second month of age in the treated groups compared to control. Klawns (1985) and Lebas et al., (1986) indicated that the testicular descent into the scrotum of rabbits takes place shortly before puberty at the age of 3-4 months. This could be recognized as a sign for the steroid activity of the testis in the male before reaching the expected age of puberty in rabbits. It has long been known that the testicular descent is completed under the influence of androgens secreted by the testes (Hafez, 1986).

Carnitine has an important role in spermatogenesis regarding synthesis and protection of cell membranes (Uhlenbruck, 1996), beside storage of activated acetic acid in form of acetylcarnitine, which is an energy-rich compound that serves as

the primary energy source for sperm motility after insemination (Jeulin et al., 1988). On other hand, L-tyrosine might induce early puberty in male rabbits as a growth promoting factor (Miller, 1973) and a stimulant to the gonadotrophin releasing factor (Kamberi et al., 1971) which potentiates the testicular function. The sexual behavior of females is synchronized by the ovarian activity (Hafez, 1986), as the presence of developing follicles in the ovaries is followed by secretion of estrogen resulting in the female standing position to be mated by the male. This support the hypothesis that tyrosine functions in the synthesis of cerebral catecholamine, this was shown to activate the hypothalamus to release GnRH (Hammerl and R, sse, 1987; Arthur, 1989) which stimulates the Pituitary gland to secrete gonadotrophins (FSH & LH) activating the ovaries. Kamberi et al., (1971) reported that dopamine, created from the amino acid precursor L-tyrosine, might function as a synaptic transmitter to potentiate FSH releasing factor or LH-Releasing factor or both.

It is of interest to notice that the testicular size of rabbits throughout the experimental period appeared remarkably larger in the tyrosine as well as carnitine treated groups compared to control in both ages (A and B), a finding which confirms the close relationship between the testis weight and body weight, and also the relation between the testis weight and the age of the animal (Amann, 1970). Similarly, the ovarian size showed a highly

significant increase in the tyrosine and carnitine treated rabbits when compared to the control group (Table, 3). L-carnitine was highly effective in rabbits at two and three months of age where the ovarian weight in the treated cases was higher than that of the control. On contrast with tyrosine treated females, where the ovarian size was remarkably high in ages, two and three month groups than both carnitine and control groups. Thus, if it has been supposed that puberty in rabbits takes place at four months age (Harkness and Wagner, 1989), treatment with tyrosine and carnitine promotes the developing process of gonads to module puberty at a stage earlier than that under the natural conditions.

When comparing the treatment effects on the number of birth, it reveals that in treatments groups, tyrosine and carnitine, the numbers of kids were higher than that of control in both ages. These results however differ than that of Musser et al. (1999) on pregnant sows where, they postulated that carnitine does not affect number of birth rather than litter weights and viability.

From the clinical observations and results of the present study it could be concluded that both amino acids L-tyrosine and L-carnitine could have the following physiological functions in rabbits; 1) Growth promoting factor indicated by the prominent increase in body weight and sizes of gonads. 2) Enhancement of puberty in both males

and females.

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تأثيرات اعطاء التيروسين و الكارنيتين على احداث البلوغ في الأرناب

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تمت هذه الدراسة على عدد 72 أرناب من النوع النيوزيلاندى الابيض حيث قسمت هذه الأرناب إلى مجموعتين متساويتين بكل مجموعة عدد 36 أرناب في أعمار مختلفة تبدأ من شهرين وثلاثة شهور. تم استخدام عدد 18 ذكر و18 انثى في كل مجموعه. تم اعطاء عدد 12 من الحمض الامينى التيروسين بجرعة 100 مجم/كجم من وزن الحيوان مرة واحدة وإعطاء عدد 12 من الحمض الامينى الكارنيتين بجرعة 1جم/كجم لمد اسبوعين و باقى كل مجموعة استخدم كمجموعة ضابطة وتمت الملاحظة خلال فترة التجربة و تم تدوين كافة الملاحظات و إجراء التحليل الإحصائي لها. وقد لوحظ زيادة غير معنوية في درجات الحرارة في المجموعات المعطاة التيروسين أو الكارنيتين عن المجموعات الضابطة. كما لوحظ ايضا زيادة في أوزان الحيوانات مع تقدم العمر وكانت هذه الزيادة تقريبا متساوية في الذكور والإناث و لكن في المجموعات المعطاة التيروسين أو الكارنيتين كانت الزيادة معنوية بالمقارنة بالمجموعات الضابطة. ايضا لوحظ نزول الخصيتين في الذكور ، وكذلك قبول الإناث للذكور عند عمر شهرين كان واضحا في المجموعات المعطاة التيروسين والكارنيتين. كانت هناك زيادة معنوية في أحجام الخصيتين والمبايض وخصوصا في المجموعات المعالجة وذلك بمقارنتها بالمجموعات الضابطة.

من هذه النتائج نستطيع استنتاج أن الحمضين الاميين يعملان كعامل محفز للنمو و كذلك لافراز الهرمونات. و بناء على ذلك يمكن استخدامهما في النمو و النضج الجنسي في كل من ذكور و إناث الأرناب في مراحل مبكره من العمر.