

EFFECT OF ORAL L-TYROSINE TREATMENT DURING MID-LACTATION OF FRIESIAN DAIRY COWS ON MILK YIELD AND ITS REPRODUCTIVE PERFORMANCE

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SUMMARY

A total of 30 Friesian dairy cows aged 31-59 months with an average live body weight 502.6 kg were divided into three similar groups based upon age, live body weight and milk yield to evaluate the effect of oral L-tyrosine administration on milk yield and reproductive performance of Friesian dairy cows at day 121 or 140 post calving (during mid-lactation). Cows in the first group (G1) were fed concentrate feed mixture (CFM) and considered as a control group. While, those in the 2nd (G2) and 3rd (G3) groups were received an oral dose of 50 g/cow L-tyrosine at day 121 and 140 of postpartum, respectively. Results revealed that L-tyrosine treatment significantly ($P < 0.05$) increased the average daily milk production during the 2nd four months of lactation in term of actual milk yield (AMY), 4% fat corrected milk yield (FCMY), milk composition, and decreased the number somatic cell count (SCC) in milk compared to the that of the control one. L-tyrosine treatment was also improved reproductive performance and overcomes reproductive problems associated with ovarian activity such as incidence of estrus, interval from calving to the first estrus, postpartum first service, days open, number o.

f services per conception and finally improved conception rate. **In conclusion:** based upon the results obtained in this study, L-tyrosine could be used for treating cows during postpartum periods to improve cow's reproductive performance by overcoming their reproductive problems and enhancing milk production and its composition.

Keywords: *Friesian cows, L-tyrosine, milk yield, progesterone, reproduction.*

INTRODUCTION

Some authors suggested the use of some organic substances possessed to improve the productivity and reproductive efficiency of animals through enhancing the release of sex hormones (Abu El-Hamd and Sayah, 2015). One of these substances is L-tyrosine, which is a semi essential amino acid involved in the formation of catecholamine from adrenal gland and thyroxin from thyroid gland (Hammerl and Russe, 1987a). Tyrosine serves as a precursor for the synthesis of dopamine, norepinephrine, and epinephrine and is a specific brain neurotransmitter implicated in the control of GnRH and LH pulses and LH surge (Ramirez *et al.*, 1984). The treatment by some amino acids, especially tyrosine led to improve significantly reproductive and productive performance (El-Amrawi, 2008).

These amino acids may partly mediate the effect of dietary protein on the metabolism of protein, lipids and glucose, fertility, growth and production performance; and health of animals (Kim *et al.*, 2007). The amino acid tyrosine, a blood-borne metabolite, may be involved in stimulating GnRH release because availability of tyrosine influences synthesis of norepinephrine (Gibson and Wurtman, 1986 and Acworth *et al.*, 1988), a neurotransmitter that stimulates GnRH release (Ramirez *et al.*, 1984 and Terasawa *et al.*, 1988). The relationship between tyrosine and reproduction has been reported by many authors. In dairy cows, the anestrous animals (no palpable ovarian structures or detected estrus by 60 to 150 d postpartum) had lesser concentrations of tyrosine in blood plasma than estrus-cycling controls (Hammerl, 1986). Also, it was noted that more than 85% of anestrous dairy cows expressed estrus within several days after tyrosine treatment and continued to have normal estrous cycles (Munsterer, 1987). Moreover, El-Amrawi, *et al.* (1992) postulated that a single oral dose (100 mg/kg/LBW) of L-tyrosine could induce the puberty in delayed pubertal buffalo heifers. In addition, Gabr (2009) found that the double oral dose from L-tyrosine containing 100 mg/kg live body weight (LBW) at 15 day-interval induced early puberty of rabbit bucks. In cows, exogenous tyrosine increased

LH pulse frequency in growth-restricted lambs (Hall *et al.*, 1992), induced follicular growth, estrus, and ovulation in anovulatory dairy cows (Hammerl, 1986 and Munsterer, 1987) and improved expression of estrus (Hammerl and Russe, 1987b). Therefore, the aim of current work was to study the effect of oral single dose of L-tyrosine (50 g/cow) at day 121 or 140 post calving (mid-lactation) on milk yield, its composition and reproductive performance of Friesian dairy cows.

MATERIALS AND METHODS

The present study was carried out at Sakha Animal Production Research Station, belonging to the Animal Production Research Institute, Agricultural Research center, Ministry of Agriculture, during the period from July to November 2014.

Animals:

A total of 30 Friesian cows averaging 502.6±23 kg live body weight (LBW), ranging between 31-59 months of age and 1-3 parities was used in this study. All cows were chosen at mid-lactation, 121 and 140 days post calving. At the beginning of the experimental period, the experimental cows were divided into three similar groups of 10 cows each according to their LBW, parity and milk production. Cows in the 1st group were fed a basal diet without treatment and considered as a control group (G1), while cows in 2nd (G2) and 3rd (G3) groups were received an oral dose from L-tyrosine (50 g/cow) on day 121 and 140 of postpartum period, respectively. All cows were free of any diseases with healthy appearance and housed in separated groups under semi-open sheds.

Feeding system and management:

Throughout the experimental period, cows in all groups were fed equal amounts of a diet containing concentrate feed mixture (CFM), rice straw (RS) and corn silage (CS) according to the recommendation of the NRC (2001) allowances for dairy cows based on live body weight and milk yield. The CFM was composed of 37.5% yellow corn, 20% soybean meal, 15% corn gluten, 22.5% wheat bran, 3% molasses, 0.5% and 1.5% common salt. Chemical analysis of representative monthly samples of foodstuffs was analyzed for CP, CF, EE, NFE and ash on DM basis according to the official methods of the A.O.A.C. (2000). Chemical composition of CFM, RS and corn silage as well as the calculated chemical composition of the basal diet used in feeding cows in all groups are shown in Table (1).

Table (1): Chemical analysis of different feed stuffs (on dry matter basis) used in feeding cows in all groups.

Item	Chemical composition (%)		
	CFM	RS	Corn silage
DM	90.42	88.74	36.14
OM	89.54	82.83	92.4
CP	15.34	1.61	9.35
CF	11.46	37.36	17.15
EE	5.02	1.51	3.04
NFE	57.72	42.35	56.8
Ash	10.46	17.18	7.6

Experimental procedures:

Milk yield and composition:

According to the managerial practices applied in the farm, cows were milked twice daily at 6:00 and 17:00 h by milking machine. Daily milk yield was individually recorded for morning and evening milkings for the 2nd four months of lactation after treatment. Individual milk samples were monthly collected for determining milk composition using Milko-Scan (Model 133B) and somatic cell count using a Fossomatic 360 (Foss Electronic, Slangerupgade, Denmark).

Blood sampling:

Blood samples were collected with anticoagulant (heparin) from the jugular vein of each animal in all groups at 3-4 day-interval starting from treatment up to conception. Blood samples were centrifuged at 3000 rpm for 10 minutes to separate blood plasma which stored at -20°C for determination of progesterone (P4) concentration in blood plasma.

Detection of estrus and insemination:

At the beginning of 10 days of postpartum, an infertile bull (teaser) was introduced to cows of each group for 20 minutes three times daily at 6 a.m., 12 p.m. and 3 p.m. to detect the onset of the 1st estrus. Estrus was recorded in cows receptive for teaser and stood for mounting by the teaser. Cows being in heat were artificially inseminated. Number and length of estrous cycles from calving up to conception were recorded. Average concentration of P4, P4 peak and interval to P4 peak during the estrous cycle were determined. Also, post-treatment 1st ovulation, 1st estrus and intervals post-treatment, number of services per conception (NSC), days open (DO) and conception rate (CR %) were recorded. Conception was performed by rectal palpation on day 60 post-insemination and was indicated by P4 concentration on day 14 post-insemination in non-return cows.

Progesterone assay:

Direct radioimmunoassay technique (RIA) was performed for determination of plasma progesterone concentration using antibody-coated tubes kit (Diagnosis systems, laboratories Texas, USA) according to the procedure outlined by the manufacture. According to the manufacture's information, the radioimmunoassay of progesterone is a competition assay. Sample and standards are incubated with ¹²⁵I-labeled progesterone, as tracer, in antibody-coated tubes. After incubation the content of tubes is aspirated and bound radioactivity is measured. A calibration curve is established and unknown values are calculated by interpolation from the curve. The standard curve of progesterone concentration ranged from 0 to 2.4 and 0 to 2.0 ng/ml. The intra-and inter assay coefficient of variation were 5.4 and 9.1%, respectively.

Statistical analysis:

The obtained data were statistically analyzed according to Snedecor and Cochran (1980) using programm of SAS (2004). The significant differences among treatment groups were tested using Duncan's Multiple Range Test (Duncan, 1955). The statistical model used was:

$$Y_{ij} = U + A_i + e_{ij}$$

Where, Y_{ij} = Observed values, U = Overall mean, A_i = Experimental group, e_{ij} = Random error

The level of statistical significance was set at $P < 0.05$.

RESULTS AND DISCUSSION

Milk yield:

Results presented in Table (2) revealed that L-tyrosine treatment significantly ($P < 0.05$) increased average daily milk production during the 2nd four months of lactation in term of actual milk yield (AMY) by 14.48 and 15.17%, respectively. Whereas, it was increased based on 4% fat corrected milk yield (FCMY) by 12.59 and 17.04% in G2 and G3 cows as compared to the control one. It was interested to note that L-tyrosine treatment at day 140 postpartum (G3) significantly ($P < 0.05$) increased percentages of fat, protein and lactose as compared to G1 (3.65, 3.07 and 4.16 % vs. 3.54, 2.75 and 4.00%), respectively. However, the treatment with L-tyrosine at day 121 postpartum (G2) significantly ($P < 0.05$) increased only percentages of protein and lactose by 2.98 and 4.23% as compared to G1 (Table 2). The increase obtained in AMY for cows in G2 and G3 as a results of L-tyrosine treatment reflected in a significant ($P < 0.05$) increase in yields of protein, lactose and fat in treated groups than in untreated control cows (Table 2). Also, somatic cell count showed significantly ($P < 0.05$) lower in G2 and G3 than in G1 based upon L-tyrosine treatment (285.2 and 271.4 vs. 365.6 $\times 10^3$ /ml, respectively, Table 2) indicating the beneficial effect of L-tyrosine treatment for producing clean milk. In agreement with the marked increase in milk production of cows in treated groups (G2 and G3), Abu El-Hamd *et al.*, (2010) found that L-tyrosine treatment improved ($P < 0.05$) cow's daily milk production by 15.6% in compared with that of untreated control cows. In addition, L-tyrosine treated groups had a

higher ($P < 0.05$) percentages of fat and protein as compared to the control. Similarly, Rae and Ingalls (1984) found that oral administration of tyrosine to dairy cows increased milk production by 1.0 kg/day. However, there was no effect on FCMY yield, milk composition and milk fat yield, but yields of milk protein and lactose were increased by tyrosine supplementation. This is mostly attributed to the increase in blood supply (Mephram, 1982) and the energy intake of mammary gland cells (Anderson, 1979 and Wurtman, 1982) in addition to its effect on increasing growth hormone via its effect on brain catecholamine's (Martin, 1980) in particular norepinephrine who are believed to be potent stimulators of growth hormone (GH) release. (Wurtman, 1982). This explanation is supported by the previous findings of Machlin (1973) and Peel et al. (1981) who found that administration of exogenous growth hormone increases milk production.

Table (2): Yield and composition of milk produced by cows in different experimental groups.

Item	Experimental group			±MSE
	G1	G2	G3	
Average daily milk yield (kg/day):				
Actual milk yield	14.5 ^b	16.6 ^a	16.7 ^a	0.25
4% fat corrected milk	13.5 ^b	15.2 ^a	15.8 ^a	0.22
Milk composition (%):				
Fat	3.54 ^{ab}	3.46 ^b	3.65 ^a	0.038
Protein	2.75 ^b	2.98 ^a	3.07 ^a	0.045
Lactose	4.0 ^b	4.23 ^a	4.16 ^a	0.039
Component yields (g/day):				
Fat	51.33 ^b	57.60 ^{ab}	60.96 ^a	2.94
Protein	39.87 ^b	49.47 ^a	51.27 ^a	4.34
Lactose	58.0 ^b	70.22 ^a	69.47 ^a	3.21
Somatic cell count: (10 ³ /ml)	365.6 ^a	285.2 ^b	271.4 ^b	21.4

^{a and b}: Means within the same row with different superscripts are significantly different at $P < 0.05$.

On the other hand, the observed increase in percentage and yield of milk protein in treated groups may attributed to an elevated supply of tyrosine to the mammary gland, whereas plasma tyrosine is the sole precursor of tyrosine in milk protein (Mephram, 1982), so an adequate supply of exogenous tyrosine is probably essential for synthesis of milk protein. The same author also, added that there is a relationship between uptake of tyrosine by the mammary gland and its transfer into milk, suggesting that the availability of tyrosine could limit milk protein synthesis under some conditions. Moreover, Guinard *et al.* (1994) and Metcalf *et al.* (1994 and 1996) showed that when supplemental protein or amino acid are administered to dairy cows, the mammary uptake of those amino acid and extract either more peptides or free amino acid to meet the net requirements for milk protein synthesis.

Reproductive traits:

It was interested to note that the cows in both treatment groups did not exhibit estrous activity prior to L-tyrosine treatment. Data in Table (3) showed that the interval from tyrosine treatment to oestrus incidence in G2 and G3 was 6.5 and 7.13 days, respectively. The early incidence of estrus in cows treated with L-tyrosine was indicated by Wetzel (1985) who detected a Graafian follicle after three days in dairy cows treated with oral intake of a single dose of L-tyrosine (100 mg/kg LBW). Also, Munsterer (1987) recorded about 90% of cows suffering from anestrus came into heat after three to four days of treatment with 50 g L-tyrosine per cow. The positive effect of L-tyrosine on induction of estrus in anestrus buffalo cows was indicated by El- Amrawi, *et al.* (1991), who found that about 87.5% of buffalo-cows came to heat within 6-9 days after treatment with a single oral dose of L-tyrosine (100mg/kg body weight). Also, Stevenson *et al.* (1997) recorded that supplemental tyrosine tended to reduce intervals to first postpartum ovulation of cows. Furthermore, El-Amrawi, *et al.* (1992) found that about 85% of buffalo-heifers exhibited heat within 3-4 days after treatment a single oral dose of L-tyrosine (100mg/kg body weight). This means that postpartum 1st oestrus interval (PPFEI) was 126.5 days in G2 being significantly ($P < 0.05$) shorter by 31.5 and was 147.1 days in G3, being significantly ($P < 0.05$) shorter by 10.9 days than that of the control group (158.0 days). In accordance with the present results in particular for cows in G2 treated with L-tyrosine on postpartum period,

several authors reported that exogenous tyrosine induced follicular growth and estrus in anovulatory dairy cows (Wetzel, 1985; Hammerl, 1986 and Munsterer, 1987).

Table (3): Reproductive traits of cows in different experimental groups.

Item	Experimental group			MSE
	G1	G2	G3	
Interval from treatment to 1 st estrus (day)	-	6.50	7.13	1.10
Postpartum 1 st service interval (day)	158.0 ^b	126.5 ^c	147.1 ^a	1.60
Conception rate 1 st service (%)	20	60	40	
Conception rate 2 nd service (%)	20	40	20	
Conception rate 3 rd service (%)	20	-	20	
Number of services per conception	2.00 ^a	1.40 ^b	1.75 ^{ab}	0.10
Days open (day)	172.0 ^a	134.0 ^b	158.1 ^{ab}	6.10
Total conception rate (%)	60 ^c	100 ^a	80 ^b	

^{a and b:} Means within the same row with different superscripts are significantly different at ($P < 0.05$).

Moreover, Stevenson *et al.* (1997) found that tyrosine increased the percentage of cows in estrus. While in sow, exogenous tyrosine improved expression of estrus (Hammerl and Russe, 1987b). In spite of these findings, L-tyrosine treatment had a beneficial effect on the number of services per conception (NS/C) and days open (DO). L-tyrosine treatment of cows in G2 significantly ($P < 0.05$) reduced NS/C to 1.4 services and DO to 134.0 days as compared to 2.0 services and DO of 172.0 days in the control group. Also, a similar trend was recorded in G3 (1.75 services and 158.1 days, respectively), but the differences between G3 and the other two groups (G1 and G2) were not significant. More benefits were obtained from L-tyrosine treatment in terms of conception rate (CR), being significantly ($P < 0.05$) the highest in G2 (100%), the lowest in G3 (80%) and the lowest in G1 (60%). Such a trend was in agreement with the results of Hammerl (1986) who reported that the conception rate was greater (69%) in anestrus dairy cows detected in estrus after tyrosine treatment than in anestrus cows detected in estrus after receiving no tyrosine. Similarly, Abu El-Hamed *et al.* (2010) in cows, Shamiah *et al.*, (2013) in Frisian heifers and Ibrahim (2010) in sheep, found that animals treated with L-tyrosine administration had a higher conception rate in comparison with that untreated. In addition, the amino acid tyrosine, a blood-borne metabolite, may be involved in stimulating GnRH release because the availability of tyrosine influences the synthesis of norepinephrine (Wurtman *et al.*, 1981; Gibson and Wurtman, 1986; Acworth *et al.*, 1988) a neurotransmitter that stimulates GnRH release (Ramirez *et al.*, 1984; Terasawa *et al.*, 1988). In addition, El-Amrawi, *et al.* (1991) found that the CR of buffalo-cows after treatment with a single oral dose of L-tyrosine (100mg/kg body weight) at day 42 post service was 67%. Also, Aumann (1987) showed that after L-tyrosine administration in a dose of 100 mg/kg body weight to the bitches in their food at the 5th, 6th and the 7th day of pro-estrus period, a higher mounting capability and pregnancy rate were observed in treated animals than in the control one.

Ovarian activity:

Results in Table (4) showed that L-tyrosine treatment in G2 and G3 significantly ($P < 0.05$) decreased the average number of estrus cases and ovulatory cycles per cow, and P4 concentration during the ovulatory cycles in comparison with those of the control group. The decrease in the number of cycles may be due to an increase in the milk yield as a result of elevation in prolactin level which suppresses FSH release from the anterior pituitary gland. The present results also showed that P4 level significantly ($P < 0.05$) increased prior to estrus incidence in G2 and G3 as compared to G1. On the other hand, the differences in the average number of total ovulations, silent ovulations per cow, ovulatory cycle length as well as P4 peak and interval to P4 peak during the ovulatory cycles among experimental groups were not significant. The decreased average number of estrus per cow was associated with that exogenous tyrosine induced follicular growth and ovulation in anovulatory dairy cows (Wetzel, 1985; Hammerl, 1986; Munsterer, 1987, Abu El-Hamd *et al.* 2010 and Abu El-Hamd, 2013). The marked elevation in P4 concentration during the interval from treatment to estrus incidence in G2 and G3 than in G1 was in agreement with several authors. In this respect, El-Amrawi *et al.* (1991) found that P4 level in anestrus buffalo pre-treatment was lower than 0.2 ng/ml whereas, higher values for P4 (1.7 ng/ml) were estimated in animals treated with L-tyrosine (100 mg/kg body weight) than that recorded in the control one (0.16 ng/ml). Also, Abu El-Hamd (2013) and Shamiah *et al.*, (2013) found that, one oral dose of L-tyrosine induced a significant increase in P4 concentration compared to the control.

The increased level of P4 may be due to the stimulatory effect of tyrosine on GnRH. L-tyrosine may involve stimulation of GnRH release because availability of tyrosine influences synthesis of norepinephrine (Wurtman *et al.*, 1981), a neurotransmitter that stimulates hypothalamic GnRH release and pulsatile and pre-ovulatory release of LH from pituitary gland (Ramirez *et al.*, 1984 and Terasawa *et al.*, 1988). Moreover, the catecholamines may mediate effects of other neurotransmitters and gonadal steroids on release of GnRH (Ramirez *et al.*, 1984 and Yen and Vale, 1990).

Table (4): Ovarian activity and progesterone (P4) concentration of cows in different groups starting from treatment up to conception.

Item	Experimental group			MSE
	G1	G2	G3	
Total number of ovulations/cow	3.50	2.75	3.00	0.33
Number of silent ovulations/cow	0.75	0.00	1.00	0.26
Total number of estrus cases	3.25 ^a	2.50 ^b	2.25 ^b	0.19
Number of ovulatory cycles/cow	3.25 ^a	2.25 ^b	2.50 ^b	0.14
Ovulatory cycle length (day)	20.64	21.25	22.67	3.41
Average of P4 prior to estrus activity ¹	0.214 ^b	0.435 ^a	0.417 ^a	0.03
Average P4 concentration (ng/ml) ²	2.601 ^a	1.841 ^b	1.739 ^b	0.21
P4 peak (ng/ml) ²	7.190	6.221	5.701	0.74
Interval to P4 peak (day) ³	10.14	11.63	11.56	2.64

¹ From time of treatment to estrus incidence.

² During the ovulatory cycles.

³ From the beginning of the ovulatory cycle.

In conclusion: Upon the results obtained and mentioned above, L-tyrosine is very useful and could be used for treating cows after calving for overcoming reproductive problems associated with ovarian activity and some reproductive traits such as incidence of estrus, interval from calving to the first estrus, postpartum first service, days open, number of services per conception to improve conception rate, and finally improve cow's reproductive performance, milk yield and its composition.

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تأثير المعاملة بالتيروسين بطريق الفم في منتصف موسم الحليب لأبقار الفريزيان الحلابة علي إنتاج اللبن والكفاءة التناسلية

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تم تقسيم 30 بقرة فريزيان حلابة عمرها من 31-59 شهر ومتوسط وزن الجسم الحي 502.6 كجم إلي ثلاثة مجموعات متشابهة علي أساس العمر ووزن الجسم الحي وإنتاج اللبن لتقييم تأثير المعاملة بالـ L-tyrosine عند اليوم 121 أو 140 بعد الولادة (منتصف موسم الحليب) علي إنتاج اللبن والكفاءة التناسلية لأبقار الفريزيان الحلابة. غذيت المجموعة الأولى (ج1) علي مخلوط عليقة مركزة واعتبرت كمجموعة ضابطة (كنترول) بينما استقبلت المجموعتين الثانية (ج2) والثالثة (ج3) جرعة عن طريق الفم من الـ L-tyrosine قدرها 50 جم/بقرة عند اليوم 121 واليوم 140 من فترة ما بعد الولادة. أوضحت النتائج أن المعاملة بالـ L-tyrosine حسنت معنوياً ($P < 0.05$) من متوسط إنتاج اللبن اليومي ومكوناته أثناء الأربعة شهور التالية من المعاملة علي أساس الإنتاج الفعلي للبن وعلي أساس نسبة الدهن المعدلة إلي 4% كما خفضت المعاملة من عدد الخلايا الجسدية باللبن مقارنة بالمجموعة الضابطة أو الكنترول. أدت المعاملة بالـ L-tyrosine إلي تحسن الكفاءة التناسلية للأبقار وحل المشكلات التناسلية المتعلقة بنشاط المبيض مثل إحداث الشياخ؛ الفترة من الولادة حتي ظهور أول شياخ وأول تلقحة. الأيام المفتوحة، عدد التلقيحات لكل حمل وفي النهاية تحسن نسبة الحمل. **الخلاصة:** إستناداً إلي النتائج المتحصل عليها في هذه الدراسة يمكن إستخدام الـ L-tyrosine لمعاملة الأبقار في فترة ما بعد الولادة للتغلب علي مشاكلها التناسلية وتحسين كفاءتها التناسلية وأنتاج اللبن وتركيبه.