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REPRODUCTIVE AND PRODUCTIVE PERFORMANCE OF DAIRY COWS AFTER LAMENESS TREATMENT

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ABSTRACT

Lameness in dairy cattle is a significant problem in intensive dairy industries around the world, causing production losses. This trial was undertaken to determine some reproductive and productive parameters of dairy cows after lameness treatment to anticipate their future performance. Twenty Holstein dairy cows were divided into: Ten healthy cows used as a control group and ten cows after treatment of lameness as treated group. Serum samples at the first estrous detection were collected for biochemical analysis. The results showed significant increase $P<0.05$ in serum nitric oxide level (NO), lysozyme activity (LA), Serum progesterone (P4), total protein (TP) and globulin (GL) concentrations in the treated group compared to the control one. Also, there was significant increase $P<0.05$ in the days open, days to first estrous detection, days to 1st insemination and number of services\conception in the treated group compared to the control group. The body weight (BW) has a significant decrease $P<0.05$ in the treated than the control cows after one month post-partum. The milk yield in 1st and 2nd month's, Milk fat at 4th, 5th and 6th months and Milk lactose at 5th month post-partum showed significant decrease $P<0.05$ in the treated group compared to the control group. In conclusion, lameness despite treatment somewhat disturb reproduction and milk production even if the locomotion score was apparently normal so, our decision should depend on benefit risk ratio and individually in each case.

Key words: *lameness treatment, stress, post-partum period, reproduction, lysozyme, nitric oxide, biochemical, hormonal profile.*

INTRODUCTION

Lameness in cattle is one of the major issues in modern dairy production. It does not only impair performance, but it is also, a prominent significant welfare concern in the dairy industry (Adams *et al.*, 2016); and to the individual cow (Greenough *et al.*, 1997). Lameness is one of the most costly dairy cow diseases and it was the third most frequent reason for culling after infertility and mastitis (Schindhelm *et al.*, 2017), yet adoption of lameness prevention strategies remains low (Dolecheck and Bewley, 2018), causing stress, poor reproductive performance, production losses (Walker *et al.*, 2008; Huxley, 2013 and Stankov, 2014). Stress is known to have a negative impact on reproductive hormones from the hypothalamus-pituitary-ovarian axis (Dobson *et al.*, 2003).

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After calving lameness resulted in inferior fertility that the cow was less likely to conceive, delayed its ovarian cyclist and increased the incidence of ovarian cysts compared to healthy cows (Melendez *et al.*, 2003 and Gabarino *et al.*, 2004). Green *et al.* (2002) estimated the reduction in milk yield if the cow became lame. However, Hultgren *et al.* (2004) also identified cows with a higher yield than average to be more prone to develop lameness compared to cows with a lower milk yield. Rajala-Schultz *et al.* (1999) were reported that cows treated for foot and leg problems in the beginning of lactation had a greater risk of being culled than healthy cows.

While recent work suggests early treatment of lame cows is fundamental in promoting recovery and reducing recurrence of cases (Groenevelt *et al.*, 2014 and Leach *et al.*, 2012). After lameness treatment, locomotion score was significantly improved when compared with the previous time-point. Thus, in these cows, the deleterious effects of lameness persisted for a longe time, despite treatment (Laven *et al.*, 2008).

Our study aim to throw some light on the reproductive and productive performance parameters for lactating cattle after lameness treatment which may be helpful to lamed dairy animals decision making process.

MATERIALS AND METHODS

1 Animals:
Twenty (n=20) Holstein dairy cows aged between (4-5years) weighing between (500-650 kg) in a private farm were divided into two groups. Based on Sprecher *et al.* (1997) lameness scores, animals were grouped as either cow with an average (score of ≥ 2) which was considered to be lame and after treatment they served as treated group or non-lame (score of 1) which consider as control group.

All cows were fed the same diet and offered as a total mixed ration. The diet was formulated to meet the nutritional requirements of cows according to (NRC, 2001) and offered for ad libitum consumption to leave 3-5 % daily feed refusal, also cows were given ad libitum access to water. Body condition score (BCS) was assigned by (1-5 grades) using the visual technique developed by (Edmonson *et al.,* 1989).

2- Lameness treatment:
Lameness occurred after about (2-3weeks) post calving; lamed cows were suffering from laminitis as a consequence of postpartum sub-acute ruminal acidosis (SARA) are probably coming from a sudden shortage of fiber sources after calving.

Lame animals were provided deep bedding (straw or sand) until the time at which they are better able to move and bear weight on the affected limb. The treatment was topically by non-steroidal anti-inflammatory drugs (NSAIDs) as a transdermal Flunixin solution (3.3 mg/kg) BW for the control of pain and with a combination of (potassium iodine + zinc oxide) and copper sulfate as a foot bath should be placed outside the milking parlor so that the cows dip their feet as they enter or leave the milking parlor. An antibiotic (Cefazolin) used if there was a claw ulcer, the topical paste was applied immediately after claw debridement to 10 cm then covered using a light hoof elastic bandage. The treatment was repeated one more time during the 1st week. We considered to be cured once a complete epithelial layer had been established on the lesion and they were not painful upon palpation which occurs about (2-3) weeks post-treatment. An assessment of locomotion score was made after treatment and it was significantly improved when compared with the previous time-point.

3 - Samples
Blood samples were obtained from the coccygeal vein once after treatment of lameness surrounding the first observed estrus following the resumption of ovarian cyclist after calving. Blood samples were collected into 10 mL vacutainer tubes and allowed to clot and kept at 4 °C until separation of serum. Clotted blood was centrifuged at 3000 rpm for 20 min. Serum samples were stored at −20 °C until analysis. Determinations of some biochemical parameters were done spectrophotometry by commercial kits as, total protein (Gornal *et al.*, 1949), albumin (Dumas *et al.*, 1971) and calculated globulin. Serum hormone concentrations were measured by colorimetric competitive enzyme-linked immunoassay (ELISA) technique; progesterone according to (Autrere and Benson., 1976). Cortisol according to (Ruder *et al.*, 1972). Biomarkers of oxidative stress; UV (Ultra Violet) method for glutathione peroxidase (GPX) (Pagila and Valentine 1967) and Nitric oxide (NO) (Rajaraman *et al.*, 1998). Malondialdehyde (MDA) by colorimetric method (Ohkawa *et al.*, 1979) and the serum lysozyme activity (LA) Schltz, (1987).

Milk sampling and analyses: Cows were milked twice daily at 0500 and 1600 h, on 1st, 2nd, 3rd, 4th, 5th and 6th months relative to parturition. Milk quantities were calculated as milk yield. The samples were biochemically analyzed to estimate protein, fat and lactose using Lactoscan SLP (Bulgaria).

4- Reproductive performance parameters:
Cows were synchronized using PGF2á and GnRH protocol. Conceptions were confirmed by Veterinary Ultrasound Scanner using a 7.5 MHz linear array probe (Dynamic Imaging, Livingstone, UK) after about (45-60) days of AI. Days open, days to 1^{st} estrous detection, days to 1^{st} insemination and number of services per conception were recorded as they are good index for reproductive efficiency Hegazy and EL-Ekhnawy, (2002) and Billings, (2002).

5 Statistical Analysis:
Data was collected and analysis was carried out using one way ANOVA test, and differences between median and range at P<0.05 were considered significant according to Snedecor and Cochran, (1989).

RESULTS

The present results showed non-significant differences P<0.05 in serum progesterone and cortisol levels between treated and control groups, table (1).

Table (1): Serum progesterone and cortisol concentrations in the control and treated groups surrounding the first observed estrus.

Parameters	Control group	Treated group
Progesterone (ng/ml)	0.25 ± 0.20	0.29 ± 0.14
Cortisol (ng/ml)	5.81 ±0.50	6.09 ±0.80

*:significant increase at P<0.05

Protein profile showed significant increase P<0.05 in total protein and globulin levels in the treated group compared to the control (7.83 ± 0.027, 6.94 ± 0.03 and 3.64 ± 0.013, 2.71 ± 0.033 respectively). Albumin levels showed non significance difference between studied groups, table (2).

Table (2): Serum total protein, albumin and globulin concentrations in control and treated groups surrounding the first observed estrus.

Parameters	Control group	Treated group
Total protein (gm/dl)	6.94 ± 0.03	7.83 ± 0.027*
Albumin (gm/dl)	4.23 ± 0.02	4.19 ± 0.01
Globulin (gm/dl)	2.71 ± 0.03	3.64 ± 0.013*

*:significant increase at P<0.05

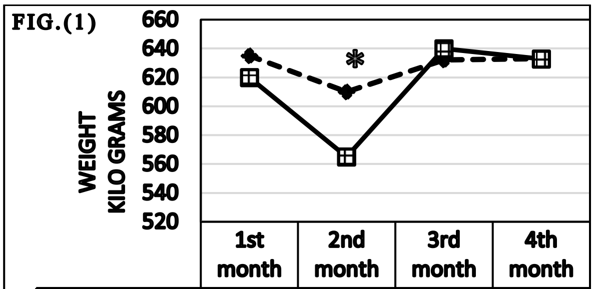
The results showed significant increase P<0.05 in NO and LA levels in the treated group compared to control (78.04 ± 7.78, 52.44 ± 3.09 and 99.44 ± 6.41, 65.43 ± 6.11 respectively), while there was no significant differences in serum GPX peroxidase and MDA concentrations between the studied groups, table (3).

Table (3): Serum glutathione peroxidase, serum nitric oxide, lysozyme activity and malondialdehyde Levels in the control and treated groups surrounding the first observed estrus.

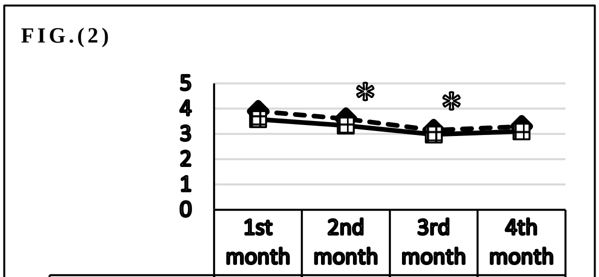
Parameters	Control group	Treated group
Glutathione peroxidase (GPX) (Mu\ml)	1.05 ± 0.23	1.6 ± 0.30
Nitric oxide (NO)(Umol\L)	52.44 ± 3.09	78.04 ± 7.78*
Lysozyme activity (LA)(Ug\ml)	65.43 ± 6.11	99.44 ± 6.41*
Malondialdehyde (MDA)(nmol\ml)	27.31 ± 1.50	25.63 ± 2.60

*:significant increase at P<0.05

Concerning the body weight the results showed significant decrease P<0.05 in the treated group compared to the control at two months post-partum (565 ± 5.1, 610 ± 5.5 respectively), Fig. (1). The body condition score of the studied groups in the four months after calving demonstrated in Fig. (2). There was significant decrease P<0.05 in the treated group than the control one in the 2nd and 3rd months (3.33 ± 0.03, 3.6 ± 0.02 and 2.98 ± 0.02, 3.15 ± 0.02 respectively).



	1st month	2nd month	3rd month	4th month
control group	635	610	632	633
treated group	620	565	639.9	632.8



	1st month	2nd month	3rd month	4th month
control group	3.9	3.6	3.15	3.3
treated group	3.58	3.33	2.98	3.1

*: Significant increase at P<0.05

Figure (1&2): Body weight and body condition score in control and treated groups through the four months postpartum.

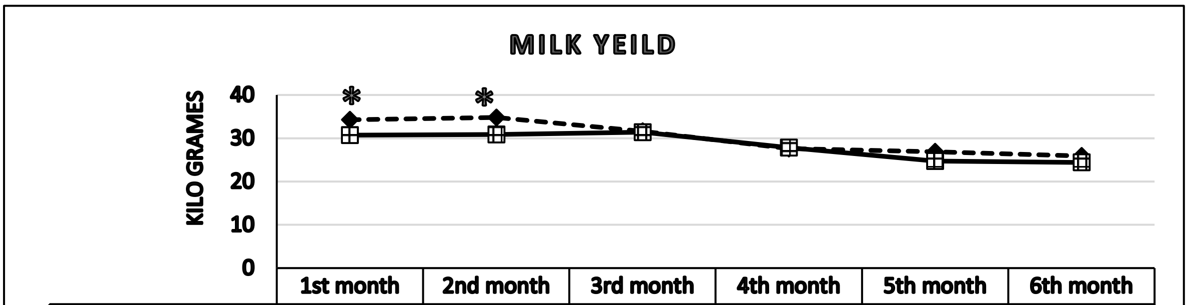
There were significant increases P<0.05 in the treated group compared to the control in the days open, days to first estrous detection, days to 1st insemination (170.2 ±4.2, 95.5 ± 3.51 and 46.3± 1.25, 29.2± 1.71 and 73.5± 2.6, 59.8± 0.4) respectively. Also, the number of services\conception showed significant increase P<0.05 in the treated group than the control (3.1± 0.13, 1.8 ± 0.06 and 1.9 ± 0.07, 2.0 ± 0.04 respectively). Lactation numbers (parity) showed non-significant difference between studied groups, Table (4).

Table (4): Reproductive performance parameters in the treated and control groups.

Reproductive parameters	Control group	Treated group
Days open	95.50 ± 3.51	170.20 ± 4.20*
Days to 1st detected estrus	29.20 ±1.71	46.30 ±1.25*
Days to 1st insemination	73.5 ± 2.60	59.80 ± 0.40*
the number of services\conception	1.80 ± 0.06	3.10 ± 0.13*
Lactation numbers	2.00 ± 0.04	1.90 ± 0.07

*: significant increase at P<0.05

The average milk yield showed significant decrease P<0.05 in the treated group compared to the control group in 1st and 2nd months post-partum (30.7 ± 0.3, 34.3 ± 0.3 and 30.9 ± 0.3, 34.8 ±0.3 respectively), Fig (3).

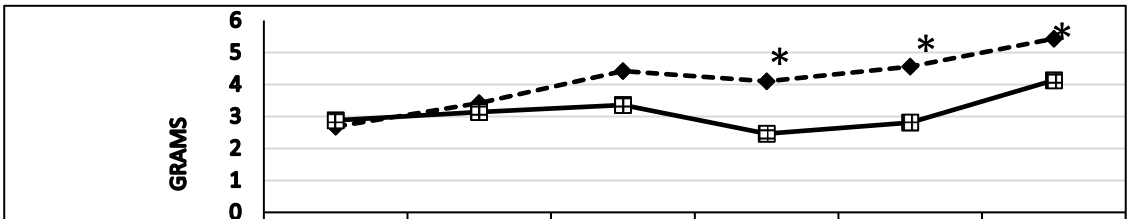


	1st month	2nd month	3rd month	4th month	5th month	6th month
control group	34.3	34.8	31.6	27.6	26.9	25.9
treated group	30.7	30.9	31.4	27.8	24.7	24.4

*: significant increase at P<0.05

Figure (3): Average milk yield in the control and treated groups through six months postpartum.

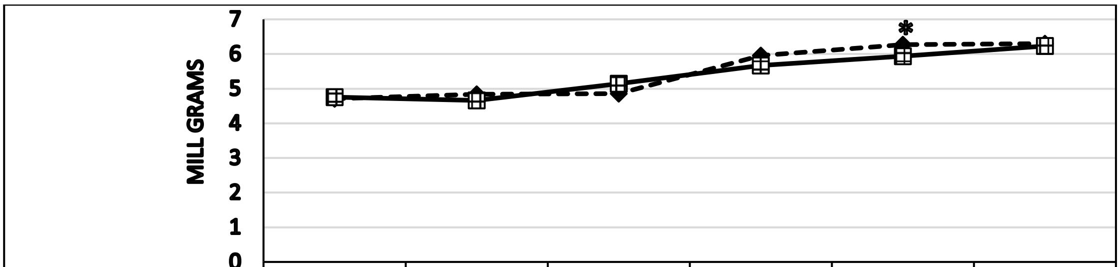
Milk fat demonstrated significant increase P<0.05 in the control group compared to the treated group in 4th, 5th and 6th months (4.1 ± 0.004, 2.46 ± 0.06; 4.56 ±.05, 2.81± 0.1, and 5.43± 0.004, 4.13 ± 0.001, respectively), Fig.(4), and in milk lactose at 5th month postpartum (6.27 ± 0.03 and 5.94± 0.03 respectively), Fig. (5). There were non-significant differences in milk protein between studied groups, Fig. (6).



	1st month	2nd month	3rd month	4th month	5th month	6th month
control group	2.68	3.42	4.42	4.1	4.56	5.43
treated group	2.88	3.14	3.36	2.46	2.81	4.13

*: significant increase at P<0.05

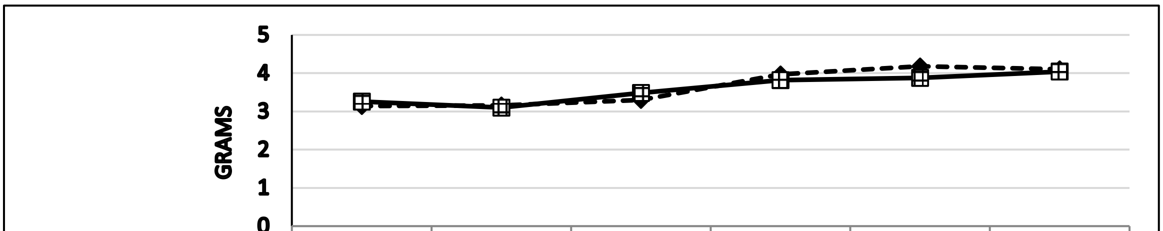
Figure (4): Milk fat in the control and treated groups through six months postpartum.



	1st month	2nd month	3rd month	4th month	5th month	6th month
control group	4.71	4.84	4.86	5.96	6.27	6.3
treated group	4.76	4.66	5.14	5.67	5.94	6.23

*:significant increase at P<0.05

Figure (5): Milk lactose in the control and treated groups through six months postpartum.



	1st month	2nd month	3rd month	4th month	5th month	6th month
control group	3.14	3.16	3.3	3.97	4.18	4.1
treated group	3.26	3.1	3.48	3.82	3.88	4.04

*:significant increase at P<0.05

Figure (6): Milk protein in the control and treated groups through six months postpartum.

DISCUSSION

Despite the fact that thousands of lamed animals are diagnosed and treated for these conditions every year, experimental evidence is limited to the further production and reproductive potential after different treatment protocols Thomas *et al.* (2015). There are insufficient data that give insight and valuation to the lamed treated animal's performance. Therefore, we have relied as much as possible on existing studies available on laminated cows.

The present results showed non-significant differences P<0.05 in serum progesterone and cortisol levels between treated and control groups, table (1). The obtained results agreed with Morris *et al.* (2013) in progesterone where they concluded that lamed cows with or without a high somatic cell count did not affect progesterone profile once ovarian cyclicity had been resumed in dairy cows. On the other studies (Walker *et al.*, 2008) on spontaneously cyclic lame cows, progesterone concentrations were lower in the late luteal period, the low progesterone concentrations in chronically stressed cows may be due to changes in the function of the hypothalamic-pituitary-adrenocortical axis (HPA). Also, Serum cortisol concentration showed non-significance difference between studied groups. This result coincided with that reported by Ley *et al.* (1996) and Walker *et al.* (2008) where they found that lameness is not associated with elevated resting cortisol concentrations. The normal cortisol level in lammed treated group denoted reduction of stress after treatment or may be the result of habituation to the same constant stress of lameness Ostrander *et al.* (2006).

Protein profile showed significant increase P<0.05 in total protein and globulin levels in the treated group compared to control (7.83 ± 0.027, 6.94 ± 0.03 and 3.64 ± 0.013, 2.71 ± 0.033 respectively). These results agreed with Tóthová *et al.* (2017) and Jawor *et al.* (2008) they reported a significant increase in serum total proteins and globulin levels in cows suffering from hoof and limb inflammatory diseases compared to control. Our results showed non-significant difference between studied groups in serum albumin level. In contrast, Yaylak *et al.* (2009) recorded that cows with a lameness score of 4 significantly showed lower concentrations of albumin, these differences might be caused by the severity degree of the disease and the treatment of the cases.

The results showed significant increase P<0.05 in NO and LA levels in the treated group compared to control (78.04 ± 7.78, 52.44 ± 3.09 and 99.44 ± 6.41, 65.43 ± 6.11 respectively), These results may be due to the mononuclear cells, such as macrophages, lymphocytes and plasma cells, are the most numerous cells in the chronic inflammatory diseases. Macrophages secrete many substances either constitutively or after stimulation Rappolee *et al.* (1988). Amongst these substances are different proteolytic enzymes as lysozyme intermediates which are important for the host defense (Yanni *et al.,* 1994). Nitric oxide is a cytotoxic factor released by a variety of cells. It is generated from the terminal guanidine nitrogen atom of L-arginine by NO synthase (Stuer *et al.,* 1999). Despite its role in the primary defense against bacteria, viruses and parasites (Akaike *et al.,* 1996) it has also been reported to be immunosuppressive on the immune system (Albina et al., 1991). Therefore, NO may be protective or hazardous for mammalian tissues depending on its concentration (Zelnickova *et al.,* 2008).

While there was non-significant differences in serum GPX peroxidase and MDA concentrations between the studied groups, table (3) these results disagreed with Xue-Jun *et al.,* (2015) whom mentioned that there were elevations of the serum MDA and GSH levels revealed in lamed cows compared to healthy cows. On the other hand, Al-Qudah and Ismail, (2012) reported that the level of reduced glutathione was significantly decreased in affected cows compared to healthy ones. The difference in our results may be due to treatment of lame cases as fast as they detected and that could prevent disturbance of antioxidant status.

Concerning the body weight the results showed significant decrease P<0.05 in treated group compared to control at two months post-partum (565 ± 5.1, 610 ± 5.5 respectively), these results agreed with Schindhelm *et al.* (2017) whom referred the obtained results to that the lame animals spent less time feeding than the sound animals which can be explained by the fact that feeding is done while standing, when the whole weight of the cow is bearing on her feet. Subsequently, if a cow suffers from a hoof lesion she will try to avoid standing, thus reduce her feeding time. Treatment of hoof affection may lead to decrease of pain and the weight increase again during other months. That also may explain the BCS results which demonstrated significant decrease P<0.05 in BCS in the treated group than the control one in the 2nd and 3rd months (3.33 ± 0.03, 3.6 ± 0.02 and 2.98 ± 0.02, 3.15 ± 0.02 respectively). While, there was no significant difference in the further months. These results may be due to lame cows are believed to lose body condition class over time due to changes in feeding habits or intrinsic pain affecting feed conversion Webster *et al.* (2001). Recent findings have shown that cows with low body condition are more likely to become lame Newsome *et al.* (2017); and cows with BCS < 2.5 (thin) were associated with an increased risk of lameness in the subsequent zero to two months for all cases of lameness and two to four months for claw horn lesions Green *et al.* (2014). Also, the digital cushion (DC) is not well developed in first heifers until the second and third lactations and is often depleted in thin cows with low BCS Lischer *et al.* (2002).

The current study recorded significant increase P<0.05 in reproductive parameters in the treated group compared to control in the days open, days to first estrous detection, days to 1st insemination and the number of services\conception. These results agreed with that reported by (Boissy *et al.,* 1998) whom observed that, cows treated from lameness minimize the frequency of their own mounting and the duration of being attractive to others. In addition, they may emit poorer quantity/quality of sexual pheromones or even 'stress-related' pheromones. On the other hand, (Roelofs *et al.* (2004) and Sood and Nanda (2006) reported that the reproductive performance parameters for the lame and non-lame cows are similar.

The average milk yield showed significant decrease P<0.05 in the treated group compared to control group in 1st and 2nd months post-partum (30.7 ± 0.3, 34.3 ± 0.3 and 30.9 ± 0.3, 34.8 ±0.3 respectively). This result is in agreement with Green *et al.* (2002) who concluded that clinical lameness has a significant impact on milk production. Treated cows need time to restore their milk yield capacity, this explain the reduced milk production than control in the months after lameness treatment. Also, it may be due to there is an adequate quantity and quality of food, but high yielding cows must stand for long periods to eat, and this may increase their risk of lameness again (Green *et al.,* 2002). Lucey *et al.* (1986) support also our results and reported that treatment of lameness had a less significant effect on milk yields. On the contrary, Pavlenko *et al.* (2011); Kamphuis *et al.* (2013); Toncho and Stankov (2015); and Schindhelm *et al.* (2017) disagreed with our findings and found that performance measures such as milk yield and live weight, as well as feed intake were not statistically significantly associated with lameness and couldn't proof an association.

Milk constituent as fat, protein and lactose were investigated. There were statistically decreased $P<0.05$ in milk fat content at the 4th, 5th and 6th months of lactation period in the treated group than the control group. While, milk lactose statistically decrease $P<0.05$ in the 5th month only. Milk protein not significantly differ $P<0.05$ between groups. The most substantial influence was that on milk fat content. These results agreed with Juarez *et al.* (2003) who reported lower milk fat contents in lame cows. Whereas the influence on milk lactose was less pronounced. Also, Rukol, (2012) and Amory *et al.* (2008) supported our results and suggested that lameness had a negative effect not only on the quantity, but also on the quality of dairy products. In the other hands, there are studies evidencing that lameness had only a very slight effect on the productivity of affected cows (Boelling and Pollott 1998 a; Gudaj *et al.,* 2012). These results were possibly due to the fact that lameness was more common in highly productive cows (Deluyker *et al.,* 1991) and the higher milk yields partly compensate the reduction after the occurrence of lameness.

Generally, our results detected some alterations in reproductive and productive performance parameters in treating moderate lame cows compared to control. This is supported by Laven *et al.* (2008) whom reported that the welfare impact of lameness on dairy cow is of long duration even after treated effectively because the deleterious effects of lameness remained for longer time despite the treatment. Bruijnis *et al.* (2010) also recorded that lameness in dairy cows can be a costly disease both in terms of money and cow welfare.

We can conclude that the early lameness detection and treatment make the impact of lameness minimal; Therefore, lameness despite treatment somewhat disturb reproduction and milk production even if the locomotion score was apparently normal so, maintain the lame cases (medium to severe) in the dairy herd should depend on benefit risk ratio and individually in each case. Further researches needed to identify costs of specific lameness conditions, differing lameness severity levels to provide a better lameness database that can be useful for decision making at both the herd and individual cow level to improve dairy cow efficiency.

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الكفاءة التناسلية والإنتاجية للأبقار الحلوب بعد معالجة العرج

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يعد العرج مشكلة كبيرة لقطعان الابقار الحلابة ويمثل تهديدا لصناعة الألبان المكثفة في جميع أنحاء العالم حيث يتسبب بخسائر انتاجية واقتصادية عالية. وقد أجريت هذه التجربة لدراسة بعض المعايير التناسلية والإنتاجية للأبقار الحلابة بعد علاجها من العرج لتوقع معدلات الاداء. تمت متابعة ٢٠ بقرة حلوبة هولشتاين قسمت لمجموعتين: عشرة أبقار سليمة استخدمت كمجموعة ضابطة وعشرة ابقار عولجت من العرج كمجموعة معاملة. تم متابعة ابقار المجموعتين وتقدير معدلات انتاج اللبن والمعايير التناسلية وتجميع عينات مصل في أول اكتشاف للشبق واجراء عدد من التحاليل البيوكيميائية. أظهرت النتائج زيادة معنوية P<0.05 في مستويات أكسيد النيتريك المصل (NO) ، نشاط الليزوزمي (LA)، البروتين الكلي (TP) وتركيز الجلوبيولين (GL) في المجموعة المعالجة مقارنة بالمجموعة الضابطة. أيضا، كان هناك زيادة معنوية P<0.05 في الأيام المفتوحة ، الفترة إلى أول اكتشاف شبق ، الفترة إلى التلقيح الأول وعدد التلقيحات اللازمة لحدوث الحمل في المجموعة المعالجة مقارنة بالمجموعة الضابطة. وانخفاض بوزن الجسم (BW) P<0.05 في المجموعه المعاملة من الأبقار بعد شهر واحد بعد الولادة. أظهر نتائج إنتاج الحليب في الشهر الأول والثاني ، ودهون الحليب في الشهر الرابع والخامس والسادس ولبن اللاكتوز في الشهر الخامس بعد الولادة انخفاضًا معنويا P<0.05 في المجموعة المعالجة مقارنة بالمجموعة الضابطة. واجمالا تعد الوقاية من العرج اهم كثيرا من العلاج لان الابقار المصابة حتي بعد علاجها تنخفض بها معدلات الانتاج والتناسل ومع هذا قرار الابقاء علي حيوانات اصيبت بالعرج في القطيع يجب دراسته من حيث المخاطر والفوائد لكل حيوان علي حدة. كما يجب اجراء المزيد من الدراسات لعمل قاعدة بيانات تساعد متخذي القرار بالمزارع لضمان اعلي كفاءة انتاجية بمزارع الالبان.

الكلمات الدالة: علاج العرج – الضغوط ومضادات الاكسدة – فترة ما بعد الولادة – التناسل بحيوان اللبن – الصورة البيوكيميائية والهرمونية – مزارع الالبان.