

EFFECT OF LONG CHAIN FATTY ACIDS ON THE PRODUCTION AND REPRODUCTIVE IMMUNITY OF HOLSTEIN DAIRY COWS

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Received: 31 December 2017; Accepted: 31 January 2018

ABSTRACT

Twenty two multiparous and primiparous Holstein cows were utilized in a completely randomized design to determine the effects of feeding diets containing linseed or cotton seed as sources of n-3 or n-6 FAs on milk production, uterine health, immunity, reproductive responses of heat stressed cows during the transition period. Dietary treatments were initiated 20-35 d prior to estimate calving date and continued till confirmed pregnancy postpartum. Diets were formulated to be isocaloric and isonitrogenous and started feeding the pre calving diets at July for the pre calving cows and the post calving diets were fed till conception. Dry matter intake (DMI) was measured and recorded, the average 1st three months postpartum milk production were calculated. Total mixed ration (TMR) ration were collected monthly, and analyzed. Milk samples were collected for analysis and measuring betahydroxybuterate (BHB), lysozyme and nitric oxide concentration. The obtained results showed no difference in the average DMI between the two groups in pre calving stage (12.5 for G1; 12.4 for G2, Kg/h/d), while, it was higher in the G1 (20.55) than in G2 (18.45) in the 1st three month post calving. Milk production was increased in G1 (36.42 ± 0.14 kg) than in G2 (34.57 ± 0.20 kg) but they are not significantly different. Also there was no significant difference between the two groups in milk analysis for protein, fat, SNF, and lactose at W2, W4 and at W7 postpartum. BHB concentration at W2 PP was significantly lower in G1 (25 ± 9.45 µg/mol.) than those in G2 (81.25 ± 9.15 µg/mol.), lysozyme at w2, w4 and w7 PP was significantly decreased in G1 (94.10 ± 30.73, 45.56 ± 9.86, 26.15 ± 11.12 µg/ml) than those in G2 (246.34 ± 6.11, 217.21 ± 9.56, 192.68 ± 11.048 µg/ml) respectively. Nitric oxide concentration was significantly decreased in G1 (47.38 ± 18.21, 18.43 ± 3.45, 14.27 ± 2.72 µm/ml) than those in G2 (91.79 ± 9.55, 64.73 ± 7.40, 42.53 ± 6.31 µm/ml) at W2, W4 and W7, respectively. Uterine cytology (neutrophil concentration) at day 40 ± 3 PP was higher in G2 than G1 but they were not significantly different. Days to 1st estrus and days to 1st insemination were significantly lower in group one (36.92 ± 0.52, 75.0793 ± 0.68) than those in G2 (86.18 ± 0.64, 88.94 ± 0.53) respectively. Days open and I.N. in G1 (172.89 ± 3.13, 3.19 ± 0.06) were lower than those in G2 (195.42 ± 2.78, 4.07 ± 0.07) but they were not significantly different. Conception rate (CR %) at 1st, 2nd and 3rd insemination was higher in G1 (10%, 50%, 20%) than those in G2 (8.33%, 25%, 8.33%) and also repeat breeder (%) was much greater in G2 (58.33%) than in G1 (30%). This study concluded that, using omega-3 FA may enhance the reproductive performance of dairy cows during summer season because of its anti-inflammatory potency, leading to a higher chance of survival of the embryo when supplemented during the periconceptual period by reducing the oxidative stress.

Key words: Omega 3, Omega 6, Immunity, Heat stress, Reproductive performance.

INTRODUCTION

Recent research has shown that Fatty acids (FAs) may modulate immune responses in several species including cows. Addition of FAs to dairy cow diets has become common practice in an effort to increase the energy density of the diet to prevent the state of negative energy balance (NEB) that usually accompanies the transition period of these animals. Development of new feeding strategies in which dietary fats influence the immune responses through the modulation of pro-inflammatory factors could contribute to attenuation of the immunosuppressive

effect caused by parturition, through improving immune functions involved in defense against pathogenic organisms (Lessard *et al.*, 2004). It is important to understand how different FAs modulate immune responses in dairy cows, in order to determine which fat isomers may be efficacious in improving the immunological dysfunction that is associated with the transition period. So we can increase the amount of milk production and enhance the reproductive performance by improving reproductive tract immunity and decreasing stress and inflammatory mediators postpartum especially during the summer season in Egypt which cause huge losses in dairy farms due to heat stress. The objective of this study to determine the effects of feeding diets containing linseed or cotton seed as sources of n-3 or n-6 FAs on uterine health, immunity, metabolic and

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reproductive responses of heat stressed cows during the transition period at hot season.

MATERIALS AND METHODS

Cows and Diets

Twenty two multiparous and primiparous (1-3 parity) Holstein cows were utilized in a completely randomized design to determine the effects of feeding diets containing linseed or cotton seed as sources of n-3 or n-6 FAs on uterine health, immunity, metabolic and reproductive responses of heat stressed cows during the transition period at hot season. A total of twenty-two multiparous cows were used for statistical analyses. Animals that were diagnosed with any condition that required administering antibiotics either pre- or postpartum were removed from the trial. The experiment was conducted at Egyptian farm (Elbaramoth dairy farm in EL-natroon valley), cows were started feeding the pre calving diet at July and then fed post calving diets till conception.

The two dietary treatments were initiated 28-35 d prior to estimate calving date and continued till conception. The 1st group fed diet contained linseed (G1, 10 cows), while the 2nd group (G2, 12 cows) fed diet contained Ca soap fat (palm oil fat origin) supplement and cotton seed whole lent. Diets were isocaloric and isonitrogenous. (Table 1).

Fat supplements and oilseeds were mixed with the concentrates and offered as a part of the total mixed ration (TMR) to experimental animals. The experimental diets were formulated to meet all the nutritional requirements according to (NRC 2001). Cows were fed diets for ad libitum consumption and given ad libitum access to water.

Pre and postpartum cows were housed in a free-stall; sand-bedded barn equipped with fans, sprinklers. Intake of DM was measured daily by subtracting the refusal from the total amount offered. All experimental cows were offered *ad libitum* amounts of TMR to allow for 3-5% refusals. Feed refusal collected each early morning and weighed. TMR ration were collected once pre calving and monthly post calving, and analyzed by wet chemistry for fat (acid hydrolysis), crude protein (CP), acid-detergent fiber (ADF), and neutral-detergent fiber (NDF). Detailed ingredients and chemical composition of the experimental diets are listed in (Table 1).

Postpartum cows were milked three times per day and milk amount for each cow were recorded at each milking. For each experimental cow, samples of milk from consecutive early morning and afternoon and evening milking were collected at the same day in weeks 2, 4 and 7 postpartum for analysis.

Table 1: Composition and proximate analysis of the pre and post calving experimented diets (As- fed basis).

Rations	G1		G2	
	Pre calving	post calving	Pre calving	Post calving
Feed Stuff (Kg/h/d)				
Corn Grain, Ground , Dry	2.57	7	2.5	7.5
Linseed	0.73	2		
Soybean, Meal, Solv, 47% protein	1.1	3	1.18	3.5
Wheat bran	0.46	1.25	0.25	0.75
Cotton seed, Whole with lint	-	-	0.85	2.5
Calcium soaps of fatty acids	-	-	0.12	0.36
Corn silage	25	16	25	15
Alfa Alfa hay	-	6	-	5.5
Rice straw	0.5		0.5	
Magnesium oxide	0.01	0.02	0.01	0.02
Sodium Bicarbonate	0.05	0.16	0.05	0.17
Mono- basic calcium phosphate	0.03	0.07	0.02	0.07
Premix ¹	0.04	0.02	0.04	0.02
NEW T-NIL®Dry ²	0.015	0.01	0.015	0.01
CP	13.1	17.8	13.1	17.8
NEL (M Cal/kg DM)		1.70		1.70
NDF (%DM)	35	28.1	36.1	28.6
ADF (%DM)	21.1	18	22.6	19.4
Forage NDF (%DM)	28.7	19.1	28.7	17.6
R:C ratio		44 :56		40:60
TDN (% DM)	74	76	74	76
E.E (% DM)	5.1	5.7	5.2	6.1

1= (Vit. A 10000000 IU, Vit D3 2500000 IU, Vit. E 35000 mg , Biotin 1000 mg , Zinc 100000 mg, Mn 80000 mg, Cu 30000 mg, I 800 mg, Co 400 mg , Se 300 mg , Caco3 to 3 kg).

2=Sorbic acid 0.05%, Citric acid 0.75%, Calcium propionate 10.5%, Copper sulphate 5%, Inactivated yeast (*Saccharomyces Cerevisiae*) 2%, Sapiolite 41.7%, Bentonite 40% .

Water mineral analysis was taken in consideration in ration calculation.

According to the official methods of A.O.A.C. (2012).

NEL (net energy for lactation), NDF (neutral detergent fiber), ADF (acid detergent fiber), TDN (total digestible nutrients), R: C ratio (roughage concentrate ratio), E.E (ether extract).

Experimental measurements and laboratory analysis:**Body weight and feed consumption**

Nutrients requirements were calculated on body weight basis and milk yield according to NRC (2001). The average dry matter intake (DMI) per head per day was measured and recorded for each group pre and post calving till 3 month postpartum.

Analysis of dietary nutrients content:

Dry samples from the experimented rations were ground with a Wiley mill (2-mm screen). Feed samples were analyzed for ether extract and crude protein according to A.O.A.C. (2012). NDF and ADF were determined according to Goering and Van Soest (1991).

Milk production and collection:

Individual milk yield was recorded daily for three months post calving. Milk samples were collected from each group at W2, W4 and at W7 post-partum, and analyzed for milk fat, protein, solids-not-fat and lactose using Milko-Scan FT 6000. Also samples were analyzed for beta hydroxyl butyrate (BHB) using a commercially available test (Carrier *et al.*, 2004), lysozyme (Schultz *et al.*, 1987) and nitric oxide measurement (Rajaraman *et al.*, 1998).

Statistical Analyses:

The various data were subjected to ANOVA procedure for a randomized complete design, Analysis of variance by Duncan's test according to Snedecor and Cochran, (1982). Least significant difference was applied to the data to test for differences between treatments using a computer program 'SPSS', Significance was declared at $P < 0.05$.

Uterine Health

An assessment of uterine cytology was conducted for each animal at 40 ± 3 DIM (Sheldon *et al.*, 2006). Cows were flushed using a 53.3 silicon Foley catheter. The vulva was cleaned with a 10% chlorhexidinediacetate solution and dried with a paper towel. The catheter was introduced through the cervix into the previously pregnant uterine horn. The air balloon was placed approximately 1 cm past the bifurcation of the uterine horn and inflated to a volume consistent with the size of the uterine horn. Sterile saline (20 mL of 0.9%) was infused into the uterine horn and aspirated back using a syringe with a Foley connector. The aspirated solution was placed into a sterile 50-mL conical tube and vortexed. A 10 micro. L sample of the solution was smeared onto a glass slide and allow to air dry. The smear was stained using the Protocol Hema 3 stain method. Slides were examined at a magnification of 40x with oil immersion and 100 total cells (including endothelial cells) were counted. Percent of neutrophils were calculated as follows:

$$\% \text{ neutrophils} = \text{total number of neutrophils} / 100$$

RESULTS

The obtained results showed no difference in DMI between the two groups in the pre calving stage (12.5 for G1; 12.4 for G2), while, the average three month DMI postpartum was higher in the G1 (20.55) than in G2 (18.45) (Table 2).

There was no significant difference in 1st 3 months average milk production (M.P)/ cow /day between the two experimented groups (Table 2), although it was higher in G1 (36.42 ± 0.14) than in G2 (34.57 ± 0.20), there was no significant difference between the two groups in milk analysis for protein, fat, Solid not fat (SNF), and lactose at week (W2, W4 and W7) postpartum.

Table 2: Effect of the experimented diets on the DMI (kg), average milk production (kg) and milk constituents.

Parameters	G1 (Mean±SE)	G2 (Mean±SE)	Sig.
Pre-calving DMI	12.5	12.4	-
Post-calving DMI	20.55	18.45	-
M.P	36.42 ±0.14	34.57± 0.20	0.33
Milk composition fat %			
W2	3.55 ±0.35	4.29±0.30	0.15
W4	3.03 ±0.12	3.36±0.36	0.31
W7	3.42±0.20	3.66±0.30	0.50
Protein (%)			
W2	4.03 ±0.13	3.69±0.33	0.49
W4	3.96±0.11	3.84 ±0.11	0.49
W7	3.73 ±0.09	3.97 ±0.11	0.13
SNF (%)			
W2	11.13±0.34	9.61 ±1.10	0.14
W4	10.93±0.27	10.59±0.29	0.43
W7	10.28±0.25	10.93±0.30	0.13
Lactose (%)			
W2	6.09 ±0.19	6.08±0.65	0.98
W4	5.97±0.15	5.80 ±0.16	0.49
W7	5.60 ±0.14	5.96±0.16	0.14

There was a significant difference ($P<0.01$) between BHB concentration in w2 postpartum (PP) in G1 (25 ± 9.45) than those in G2 (81.25 ± 9.15). Lysozyme in G1 (94.10 ± 30.73 , 45.56 ± 9.86 , 26.15 ± 11.12) was significantly ($P<0.01$) decreased compared to G2 (246.34 ± 6.11 , 217.21 ± 9.56 , 192.68 ± 11.048) at w2, w4 and w7, respectively. Also nitric oxide

concentration was significantly ($P<0.01$) lower in G1 (47.38 ± 18.21 , 18.43 ± 3.45 , 14.27 ± 2.72) than those in G2 (91.79 ± 9.55 , 64.73 ± 7.40 , 42.53 ± 6.31) at W2, W4 and W7, respectively. While uterine wash neutrophil concentration at day 40 ± 3 was higher in G2 than G1 but the difference between the two group was non-significantly different.

Table 3: Effect of the experimented diets on BHB, oxidative stress biomarkers and uterine cytology in heat stressed dairy cows' milk.

Parameters	G1 (Mean±SE)	G2 (Mean±SE)	P
BHB1 (μ g/mol)			
BHB1 (W2)	25±9.45	81.25 ±9.15	0.001
BHB2 (W7)	50±8.33	61.11 ±7.35	0.240
Lysozyme (μ g/ml)			
W2	94.10 ±30.73	246.34 ±6.11	0.000
W4	45.56±9.86	217.21 ±9.56	0.000
W7	26.15 ±11.12	192.68±11.048	0.000
Nitric oxide concentration (μ m/ml)			
W2	47.38 ±18.21	91.79±9.55	0.01
W4	18.43 ±3.45	64.73 ±7.40	0.000
W7	14.27 ±2.72	42.53±6.31	0.005
Uterine cytology	1.50 ±0.62	3.67±2.30	0.354

Days to 1st estrus and days to 1st insemination was significantly lower in group one (36.92 ± 0.52 , 75.0793 ± 0.68) than those in G2 (86.18 ± 0.64 , 88.94 ± 0.53 respectively). Days open (D.O) and insemination no (I.N) in G1 (172.89 ± 3.13 , 3.19 ± 0.06) were decreased than those in G2 (195.42 ± 2.78 , 4.07 ± 0.07 respectively) although they were not

significantly different. Conception rate (CR %) at 1st, 2nd and 3rd insemination was higher in G1 (10%, 50%, 20%) than those in G2 (8.33%, 25%, 8.33%), Meanwhile, repeat breeder (%) was much higher in G2 (58.33%) than in G1 (30%) as seen in table (Table 4).

Table 4: Effect of the experimented diets on reproductive performance of dairy cows.

Parameters	G1 Mean±SE	G2 Mean±SE	Sig.
Days to 1 st IN.	75.0793±0.68	88.94±0.53	0.041
Days to 1 st estrus	36.92 ±0.52	86.18±0.64	0.000
D.O	172.89±3.13	195.42±2.78	0.362
I.N	3.19±0.06	4.07±0.07	0.156
CR at 1st insemin.	10	8.33	-
CR at 2nd insemin.	50	25	-
CR at 3rd insemin.	20	8.33	-
Repeat breeders	30	58.33	-

DISCUSSION

The obtained results showed a non-difference in DMI between the two groups in the pre calving stage. While, the average DMI was higher in the G1 than in G2 in the 1st three months post calving. The low DMI in both groups was due to the heat stress which affects feed consumption during hot season, that effect was lower in G1 may be due to the anti-inflammatory effect of using omega 3 in G1 ration (Amaral *et al.*, 2008). Also the decrease in DMI in G2 can be explained by the higher rumen degradation and lower fat protection of cotton seed than linseed as a source of protected fat. As factors that determine if a reduction in DMI will occur due to FA supplementation include the degree of ruminal protection of the FA supplement and the amount of fiber fed in the diet. The depression in DMI due to fat feeding is due, in part, to a reduction of fiber digestion leading to prolonged ruminal fill, and decreased palatability attributed to fat supplements (Allen, 2000).

Milk production constituents

There was no significant difference in the first three months in the average milk production (M.P) /cow/day between the two experimented groups (Table 2), although it was higher in G1 than in G2, this non-significance may be due to the low cow number in each group. An increase in milk production may be due to higher by pass protein supplied from linseed diet and lower stress due to the anti-inflammatory effect of omega 3 in G1 (Amaral *et al.*, 2008). Meanwhile, there was no significant difference between the two groups in milk analysis for protein, fat, solid not fat (SNF), and lactose at week (W2, W4 and W7) postpartum.

Oxidative stress biomarkers and uterine cytology

Current study results revealed that there was a significant difference between BHB in the w2 postpartum (PP) in G1 than those in G2. The difference between the two groups may be due to higher feed intake postpartum and better energy efficiency. As shown in table 3, lysozyme at w2, w4 and w7 PP in G1 was significantly decreased than those in G2. Also its concentration was decreased by increasing days in milk postpartum, these results coincide with those reported by Pizato *et al.* (2006) and Calder, (2007) who found an effect of n-3 FAs on cells of the innate immune system explain why these FAs are considered immunosuppressive while, Thatcher *et al.* (2006) stated that the increased linoleic acid (LA) in tissues likely enhanced the immunocompetence of the animals postpartum due to increased PG production. Also nitric oxide concentration was significantly lower in G1 than those in G2 at W2, W4 and W7 respectively, and its concentration decreased by increasing lactation days. These results can be explained by Thatcher *et al.* (2006) who reported that feeding Omega-6 FA was believed to have proinflammatory and thus PGF2 α -stimulating properties rendering them extra value as "neutraceutical" early postpartum. But it also may cause immune failure during vaccination and disease infection because of high stress load and duration like in case of heat stress in summer. While, Amaral *et al.* (2008) reported that omega-3 FFA can weaken this inflammatory potency. Nitric oxide (NO) is one of the most important reactive nitrogen intermediates, which operates in a variety of tissues to regulate a diverse range of physiological processes such as inflammatory response (Dawson and Dawson, 1995). Activation of inflammatory and immune responses leads to an increase in cytokines production, which in turn can increase secretion of other molecules such as PGF2 or nitric oxide (Hansen *et al.*, 2004). The

decreased concentration of nitric oxide and lysozyme by increasing days in milk (DIM) postpartum can be explained as feed intake increased, which decrease the effect of negative energy balance post-partum, as cows postpartum were metabolically challenged as energy demands outstrip energy intake, and animals enter a state of negative energy balance (Ingvarlsen and Andersen, 2000). This triggers mainly catabolic pathways which, at the cellular level, increase the production of "reactive oxygen metabolites" (ROMs) (Bernabucci *et al.*, 2005). This (ROMs) production decreased by decreasing DIM and increasing feed intake PP. Also their concentrations were positively affected with increased milk production; cows with high milk yields have higher concentrations of oxidative stress than lower yielding animals (Lohrke *et al.*, 2004; Castillo *et al.*, 2006).

Uterine wash neutrophil concentration at day 40 ± 3 was higher in G2 than G1 but the difference between the two group was no significantly different, these results support the results reported by Amaral *et al.* (2008) who found that primiparous dairy cows fed a diet rich in n-3 FAs had lower neutrophil counts in uterine flushings collected at 40 ± 2 days postpartum compared to those animals fed a diet rich in saturated and n-6 FAs, suggesting that the animals fed the n-3 supplement had healthier uterine environments.

Reproductive parameters

Days to 1st estrus and days to 1st insemination were significantly lower in G1 than those in G2. As shown in table (4), days open (D.O) and insemination no (I.N) in G1 were lower than in G2 but they were not significantly different. Conception rate (CR %) at 1st, 2nd and 3rd insemination was higher in G1 than in G2, also repeat breeders (%) was much higher in G2 than in G1. The lower reproductive parameters in this study may be due to heat stress as the experiment start at summer. Heat stress has been suggested to affect oxidative status in dairy cows which can affect reproductive and productive performances. Evidence suggests that the effects of elevated temperatures on embryonic development involve changes in the metabolism of free radicals. Heat shock increases intracellular ROMs in cultured bovine embryos, which in turn delays or blocks embryo development (Sakatani *et al.*, 2008). Activation of inflammatory and immune responses leads to an increase in cytokines production, which in turn can increase secretion of other molecules detrimental for embryo survival and development, such as PGF2 or nitric oxide (Hansen *et al.*, 2004).

Oxidative stress can affect reproductive events through reactive nitrogen species like nitric oxide (Rosselli *et al.*, 1998). An endogenous nitric oxide system exists in the fallopian tubes (Rosselli *et al.*, 1996). Increased nitric oxide levels in the fallopian tubes are cytotoxic to the invading microbes and also may be toxic to spermatozoa (Rosselli *et al.*, 1995). In addition, nitric oxide might participate in the

regulation of uterine contraction (Norman *et al.*, 1999). This can result in decreased transport of sperm to ova or retained placenta (Miller *et al.*, 1993). The enhancement of reproductive performance in the G1 may be due to feeding omega 3 which has anti-inflammatory effect and decrease the oxidative stress and signs of inflammation around conception especially during summer, while feeding n-6 FA to dairy cows has a pro inflammatory effect and stimulates PGF2- α synthesis (Petit *et al.*, 2004). A sequential and selective feeding of extra n-6 FA around calving and of n-3 rich diets during the breeding period has therefore been proposed as an optimal reproductive management strategy in dairy cows (Silvestre *et al.*, 2011). The optimal immune response at the uterine level early postpartum should prevent endometritis while the n-3 supplementations around conception should safeguard embryo survival through sustained corpus luteum function.

CONCLUSION

It can be concluded that, the amount of milk production and the reproductive performance may be enhanced by improving reproductive tract immunity and decrease stress and inflammatory mediators postpartum especially during the summer season in Egypt which cause huge losses in dairy farms due to heat stress. Using Omega-3FA sources in dairy cow ration can weaken the inflammatory potency, leading to a higher chance of survival of the embryo when supplemented during the periconceptual period.

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تأثير استخدام الاحماض الدهنية الغير مشبعة على الانتاج ومناعة الجهاز التناسلي في الابقار الهولستين الحلابة

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استخدم في هذه التجربة عدد ٢٢ بقرة هولستين حلاب لدراسة تأثير استخدام علائق تحتوي على بذور الكتان او بذور القطن الكاملة كمصدر للاوميغا ٣ أو الاوميغا ٦ على انتاجية اللبن والحالة الصحية للرحم والمناعة والاداء التناسلي للابقار الحلاب المجهد في الفترة الانتقالية قبل وبعد الولادة. بدأت المعاملات الغذائية للابقار من ٢٠-٣٥ يوم قبل الولادة واستمرت بعدها حتى حدوث الاخصاب. تم تركيب العلائق لتكون متساوية في كل من الطاقة والبروتين بحيث تبدأ التغذية عليها ابتداء من اول شهر يوليو للابقار العشار فترة انتظار الولادة وتستمر على علائق ما بعد الولادة حتى حدوث الاخصاب ، تم حساب متوسط استهلاك المادة الجافة الماكولة قبل وبعد الولادة وكذلك حساب متوسط انتاجية اللبن اليومية لكل بقرة ولمدة ثلاث اشهر بعد الولادة ، تم اخذ عينات شهرية من العلف تام الخلط وتحليلها ، كالك تجميع عينات اللبن عند الاسبوع الثاني والرابع والسابع بعد الولاده لتحليلها ، تم عمل تحليل كامل للبن وكذلك قياس تركيزات كل من الليروزيم واكسيد النيتروجين والبيتا هيدروكسي بيوتيرات وكانت النتائج كما يلي : اظهرت النتائج عدم وجود فرق ملحوظ في استهلاك المادة الجافة الماكولة من العلف يوميا بين المجموعه الاولى (١٢.٥ كجم) والمجموعه الثانية (١٢.٤ كجم) ، فيما كانت المجموعه الاولى اعلى استهلاكا لمتوسط المادة الجافة الماكولة من العلف بعد الولاده (٢٠.٥٥ كجم) عن المجموعه الثانية (١٨.٤٥ كجم). متوسط انتاجية اللبن في المجموعه الاولى (٣٦.٤٢ كجم) كان اعلى من متوسطه في المجموعه الثانية (٣٤.٥٧ كجم) برغم عدم وجود معنوية في الفروق بين المجموعتين ، كذلك عدم وجود اختلاف معنوي بين المجموعتين في تركيزات كل من دهن ، بروتين ، الاجسام الصلبه دون الدهون واللاكتوز في اللبن عند كل من الاسبوع الثاني والرابع والسابع بعد الولاده. كذلك تركيزات BHB كانت اقل معنويا في المجموعه الاولى عند الاسبوع الثاني (25±9.45 ميكروجرام/مول) عن تركيزاتها في المجموعه الثانية (81.25±9.15 ميكروجرام/مول) ، كذلك تركيزات الليروزيم (ميكروجرام/ملل) في اللبن في الاسبوع الثاني والرابع والسابع بعد الولاده كانت اقل في المجموعه الاولى (26.15±11.12 , 45.5±9.86 , 94.10±30.73) معنويا عن تركيزاتها في المجموعه الثانيه (192.68±11.048, 217.21±9.56, 246.34±6.11) ، كما ان تركيزات اكسيد النيتروجين (ميكرومول/ملل) في اللبن في المجموعه الاولى (14.27±2.72 , 18.43±3.45 , 47.38±18.21) كان اقل معنويا من تركيزه في المجموعه الثانية (42.53±6.31, 64.73±7.40, 91.79±9.55) ، كذلك كانت نسبة النيتروفييل في غسول الرحم اقل نسبيا في المجموعه الاولى عن المجموعه الثانية وان كان الفرق بين المجموعتين لم يرقى للمعنوية ، في حين كان عدد الايام حتى حدوث اول شياح ، كذلك الايام حتى حدوث اول تلقح بعد الولاده اقل معنويا في المجموعه الاولى (75.0793±0.68, 36.92±0.52) عن المجموعه الثانيه (88.94±0.53, 86.18±0.64) ، كذلك عدد الايام المفتوحه حتى حدوث الاخصاب وعدد التلقيحات اللازمه حتى حدوث الاخصاب كانت اقل نسبيا دون معنوية في المجموعه الاولى (3.19±0.06, 172.89±3.13) عن المجموعه الثانيه (4.07±0.07, 195.42±2.78) ، كما ان نسبة الاخصاب عند التلقيحه الاولى والثانية والثالثة كانت اعلى في المجموعه الاولى (20% , 50% , 10%) عن المجموعه الثانيه (8.33% , 25% , 8.33%) ، كذلك كانت نسبة التخلفات التناسليه (اكثر من ٣ تلقحات) كانت اقل في المجموعه الاولى (30%) عن المجموعه الثانيه (58.33%). ونستخلص من هذه الدراره ان استخدام الاوميغا ٦ له تاثير مشجع على الالتهاب عكس استخدام الاوميغا ٣ التي لها تاثير مقاوم له وبالتالي تاثير مقاوم للاجهاد مما يعطي فرصه اعلى للأبقاء على حياة الجنين عند الحمل وتقليل تاثير الاجهاد الحراري ومن ثم رفع الكفاءة التناسليه خاصة اثناء فصل الصيف.

الكلمات الدالة : الاوميغا ٣ ، الاوميغا ٦ ، المناعه ، الاجهاد الحراري ، الاداء التناسلي