



# Effects of Dietary Omega-3 Fatty Acids on Reproductive Performance and Biochemical Parameters of Lactating Cows in Arid Subtropics

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## ABSTRACT

Dietary omega-3 fatty acids are a type of polyunsaturated fat known to improve production and body health in mammalian species. The present on-farm experiment was designed to evaluate the effects of omega-3 fatty acids sources, extruded flaxseed and salmate, on reproductive performance and biochemical parameters in lactating dairy cattle. Two hundred and sixty-eight lactating cows were blocked by stage of lactation and assigned to three groups, a control group and two treated groups fed diets containing salmate (25 g/head/day) and extruded flaxseed (7.0%). The formulated control, salmate and extruded flaxseed diets were isoenergetic and isonitrogenous. The diets were given to animals from three weeks pre-partum to 160 days postpartum. Reproductive performance and biochemical parameters were investigated in both control and treated groups. The results indicated earlier restoration of normal oestrous postpartum in flaxseed group (17/90, 18.89%) followed by salmate (13/87, 14.94%) and control (3/91, 3.30%) groups. Upon estrous synchronization of remaining not estrous cows, the pregnancy rates were the highest in flaxseed (72/90, 80.0%) followed by salmate (67/87, 77.01%) and control (49/91, 53.85%) groups. The values of total protein, globulin, blood urea nitrogen, beta-hydroxybutyrate, cholesterol, thyroxine, IGF and insulin were higher in extruded flaxseed group compared to salamate and control ones versus NEFA, glucose, triglycerides and cortisol values. In conclusion, supplementation of flaxseed and salmate provided beneficial roles to lactating dairy cows in relation to reproductive performances and biochemical parameters in arid subtropical conditions.

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## Authors' Contribution

MA designed the study. TA carried out the study and collected the data. AE statistically analyzed the results. AA wrote the manuscript. All authors interpreted the data, revised the manuscript, and approved the final version.

## Key words

Fatty acids, Extruded flaxseed, Salmate, Estrous, Conception, Metabolites

## INTRODUCTION

Dietary supplements to ruminant species affect both reproductive and blood metabolites (Al-Suwaiegh *et al.*, 2022). The global requirements of milk and meat are predicted to increase to 35.0% by 2030 due to increase in population. The national systems in KSA support the commercial production of meat and milk production to

overcome the increase of demands (Mohammed and Al-Suwaiegh, 2023). Therefore, several strategies have been explored a worldwide including dietary supplements that can improve body health and reproductive performance resulting in significant improvement of meat and milk production.

Lactating dairy cows undergo major endocrine and metabolic disorders during the transition period, which may have a negative impact on productive and reproductive performance (Gross and Bruckmaier, 2019; Pinedo and Melendez, 2022). Energy deficiency usually increases lipid mobilization from body reserves and intensive ketogenic and lipogenesis in the liver occur in cows after parturition and during the first couple of weeks of lactation due to increased activity of the mammary glands (Djokovic *et al.*, 2013; Pinedo and Melendez, 2022). Therefore, several strategies were applied to ruminant animals during transitional period to alleviate and overcome the negative effects.

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Feeding high protein diets increase blood urea nitrogen and consequently can reduce fertility of lactating dairy cows. On the other hand, low protein diets may have a negative impact on fertility and milk production (Underwood *et al.*, 2022) through delayed estrus and lower conception rates. Feeding flaxseed and long-chain fatty acids are reported to have major impact on ruminant animal production and reproduction as a strategy to maintain both productivity and fertility (Karageorgou *et al.*, 2023; Elbarbary *et al.*, 2023; Dere Yelken *et al.*, 2024). Quality and viability of embryos, as well as the general reproductive performance are increased through their addition into diets (Petit *et al.*, 2008; Leroy *et al.*, 2013). At early lactation, major changes occur in the animal's body metabolites (Al-Mufarji *et al.*, 2022, 2023). Additionally, major changes occur in the hormonal regulation of metabolic functions (Veshkini *et al.*, 2023). Negative energy balance (NEB) during early lactation usually leads to deterioration of organic matter metabolism and animals may depend on body reserves to meet energy demand (Ali *et al.*, 2021). The levels of triiodothyronine and thyroxine are considered indicators of adaptation to NEB until energy balance is maintained (Senosy *et al.*, 2017). Beta-hydroxybutyrate is the most important and abundant ketone body and considered the main blood indicator of ketosis and lipid mobilization in ruminants. Non-esterified fatty acid (NEFA) is also an indicator of ketosis (Senosy *et al.*, 2017, 2018). Most experiments on flaxseed were short-term trials during lactation stage; therefore, there is a need to provide more information on feeding extruded flaxseed for longer periods. The present study starts three weeks before parturition and extend to week 23 of lactation using two hundred and sixty-eight lactating Holstein cows. Reproductive performance and metabolic profiles during this long period were evaluated. The hypothesis is to shorten open days, improve reproductive performance and blood metabolites and hormones of high yielding dairy cows in arid subtropics.

## MATERIALS AND METHODS

### *Diets and animals management*

The present trial was conducted in a dairy farm located in Riyadh of KSA from June to December 2023. Two hundred and sixty-eight lactating cows were used for the trial. The cows had  $582.0 \pm 21.0$  kg of body weight and  $3.35 \pm 0.10$  of body condition score. They were divided through complete randomized design into three groups based on parity and milk yield. The cows were randomly distributed for investigating the effects of extruded flaxseed (7.0%) and salmate (25g/head/day) compared to control diet. The average relative humidity ranged from 8.0 to

94.0 and ambient temperature ranged from 31.0 to 48.0. It is important to indicate that all cows were kept under evaporative cooling system, which works during stressful condition. Animals were placed in an open lot. The period of experiment lasted six months. The control, salmate and extruded flaxseed diets were formulated to provide the nutrient requirements of lactating dairy cows according to body weight, body gain ( $0.2 \text{ kg day}^{-1}$ ), milk production and milk fat (3.5%) as recommended by NRC (2001). The cows were given fresh water and mineral mixture *ad libitum*. The diets of salmate and extruded flaxseed were weekly prepared to avoid lipid peroxidation.

### *Extruded flaxseed and salmate supplements*

Ingredients of extruded flaxseed include 85 g/kg flaxseed and 15 g/kg wheat bran (ARASCO, KSA). The salmate is dried fish oil contains 31% omega-3 and 5% Omega-6 fatty acids. The extruded flaxseed contains 28.0% fiber, 44.0 fat, 21.0% protein, 6.0% carbohydrates and 4.0% ash. In addition, the extruded flaxseed fat contains 47.0% omega-3 fatty acid and 15.0% omega 6 fatty acid.

### *Reproductive parameters*

Animals of each group were followed for estrous detection each three weeks post-calving for three consecutive days. In addition, estrus cycles were synchronized using protocol of GnRH + PGF $2\alpha$  + GnRH (Kesler, 2005). Reproductive parameters were evaluated through heat detection, open days, number of services per conception and conception rates.

### *Blood samples and analysis*

Blood samples were collected weekly from 10 cows of each group through tail vein puncture into two vacutainer tubes; one tube containing anticoagulant and the other one without anticoagulant for separation serum and plasma, respectively. Serum and plasma were stored at  $-20^\circ\text{C}$  until further analysis. The serum samples were analyzed for total protein (TP), albumen, globulin, and blood urea nitrogen (BUN) using commercial kits (Randox Lab., United Kingdom). Plasma concentrations of glucose,  $\beta$ -hydroxybutyrate acid ( $\beta$ -HBA), total cholesterol, triglyceride (TG), cholesterol, high-density lipoprotein (HDL) and non-esterified fatty acids (NEFA) were determined spectrophotometrically using commercial kits (Randox Lab., United Kingdom). Acetate was determined by colorimetric method using commercial kits (Biovision incorporated, USA). Glycerol was determined by colorimetric method using commercial kits (Cayman Chemical Company, Ann Arbor, MI, USA). Insulin was determined by indirect immunoassay technique using commercial kit (Alpco Company, NH, USA). Plasma

triiodothyronine (T3), thyroxine (T4) and cortisol levels were measured using commercial ELISA kits (Human, Germany), and micro-titrimetric plates. The assay procedures were done according to the manufacturer's instructions, and the absorbance values were done by automatic photometer.

#### Statistical analysis

Data of blood metabolites (TP, albumin, globulin, BUN, glucose, cholesterol, TG,  $\beta$ -HBA), hormones (Insulin, cortisol, T3 and T4) and reproductive performances (conception rate) were statistically analyzed by general linear model procedure of SAS (2008) using the following model;  $Y_{ijk} = \mu + A_i + B_j + AB_{ij} + E_{ijk}$ ; where  $Y_{ij}$ ,

the observation; A, the effect of factor A; B, the effect of factor B;  $AB_{ij}$ , the interaction between factor A and B;  $E_{ijk}$ , standard error. Duncan's Multiple Range Test (1955) was used to compare the means of the control and treated groups.

## RESULTS

Tables I and II shows that the effect of extruded flaxseed (7.0 %) and salmate (25 g/h/d dry protected fish oil) diets on reproductive performance, blood metabolites and hormones values of lactating dairy cows. The results support improvement of reproductive performance and body health conditions.

**Table I. Effect of salmate and extruded flaxseed diets on reproductive performance parameters of lactating Holstein cows.**

Periods	Control	Flaxseed	Salmate
No. of cows	91	90	87
Normal estrous, no. (%)	3 (3.3%)	17 (18.89%)	13 (14.94%)
Synchronized estrous, no. (%)	88 (96.70%)	73 (81.11%)	74 (85.06%)
Number of service/conception	2.52 $\pm$ 0.17	2.63 $\pm$ 0.14	2.58 $\pm$ 0.15
Open days	106.23 $\pm$ 3.91	102.22 $\pm$ 4.95	101.15 $\pm$ 4.80
Pregnancy, % (n)	53.85% (49/91)	80.0% (72/90)	77.01% (67/87)

Control group fed basal diet. Extruded flaxseed group fed isoenergetic and isonitrogenous diet containing 7% extruded flaxseed. Salmate group fed isoenergetic and isonitrogenous diet containing 25 g/head/day of dried fish oil.

**Table II. Effects of dietary extruded and salmate on blood metabolites of lactating dairy cattle.**

Items	Control	Treatment		SEM	P-value		
		Flaxseed	Salmate		Treat	Period	Treat x period
Total protein (g/dL)	6.700 <sup>ab</sup>	6.720 <sup>a</sup>	6.600 <sup>b</sup>	0.052	0.0880	<0.0001	<0.0002
Albumin (g/dL)	3.870	3.830	3.850	0.016	0.3604	<0.0001	0.3300
Globulin (g/dL)	2.820 <sup>ab</sup>	2.890 <sup>a</sup>	2.750 <sup>b</sup>	0.053	0.1137	<0.0001	<0.0008
Urea nitrogen (mg/dL)	15.20 <sup>b</sup>	16.02 <sup>a</sup>	15.75 <sup>a</sup>	0.252	0.0016	<0.0001	<0.0001
NEFA (mmol/ml)	0.400 <sup>a</sup>	0.240 <sup>b</sup>	0.380 <sup>a</sup>	0.017	<0.0001	<0.0001	<0.0001
$\beta$ HBA (mmol/ml)	0.460 <sup>b</sup>	0.560 <sup>a</sup>	0.480 <sup>b</sup>	0.010	<0.0001	<0.0001	<0.0001
Glucose (mg/dL)	63.72 <sup>a</sup>	58.54 <sup>b</sup>	59.5 <sup>b</sup>	0.437	<0.0001	<0.0001	<0.0001
Triglycerides (mg/dL)	34.29 <sup>a</sup>	18.60 <sup>b</sup>	20.02 <sup>b</sup>	0.546	<0.0001	<0.0001	<0.0001
Glycerol (mg/L)	8.830 <sup>a</sup>	5.930 <sup>b</sup>	4.360 <sup>c</sup>	0.014	<0.0001	<0.0001	<0.0001
Cholesterol (mg/dL)	152.75 <sup>c</sup>	200.18 <sup>a</sup>	170.66 <sup>b</sup>	3.488	<0.0001	<0.0001	<0.0001
HDL (mg/dL)	104.38 <sup>b</sup>	116.30 <sup>a</sup>	120.30 <sup>a</sup>	3.20	<0.0001	<0.0001	<0.0001
LDL (mg/dL)	20.16 <sup>c</sup>	57.84 <sup>a</sup>	29.28 <sup>b</sup>	0.251	<0.0001	<0.0001	<0.0001
Acetate (nmol/ml)	0.470 <sup>b</sup>	0.660 <sup>a</sup>	0.64 <sup>a</sup>	0.010	<0.0001	<0.0001	<0.0001
T4 (ng/dL)	2.200 <sup>b</sup>	2.360 <sup>a</sup>	1.980 <sup>c</sup>	0.040	<0.0001	0.0136	<0.0001
T3 (ng/ml)	0.950 <sup>a</sup>	0.950 <sup>a</sup>	0.800 <sup>b</sup>	0.008	<0.0001	<0.0001	<0.0001
Cortisol (ng/ml)	20.20 <sup>a</sup>	14.07 <sup>b</sup>	22.40 <sup>a</sup>	0.541	<0.0001	<0.0001	<0.0001
IGF (ng/ml)	3.820 <sup>c</sup>	5.830 <sup>a</sup>	4.400 <sup>b</sup>	0.089	<0.0001	0.0145	0.0220
Insulin (ng/ml)	0.410 <sup>c</sup>	0.650 <sup>a</sup>	0.540 <sup>b</sup>	0.014	<0.0001	<0.0001	<0.0001

<sup>a, b, and c</sup>, values in the same row with different superscripts differ significantly ( $P < 0.05$ ). Control group fed basal diet. Extruded flaxseed group fed isoenergetic and isonitrogenous diet containing 7% extruded flaxseed. Salmate group fed isoenergetic and isonitrogenous diet containing 25 g/head/day of dried fish oil. NEFA, Non-esterified fatty acids;  $\beta$ HBA,  $\beta$ -hydroxybutyrate acid; HDL, high-density lipoprotein; LDL, low-density lipoprotein; T3, triiodothyronine; T4, thyroxine; IGF, Insulin-like growth factor-1.

### Reproductive performance

Table I shows that extruded flaxseed and salmate diets restored earlier ovarian activity postpartum where 18.89% (17/90) and 14.94% (13/87) of cows were in normal heat compared to 3.3% (3/91) of control diet. Furthermore, the conception rate (%) of normal and synchronized estrous cows were the highest in extruded flaxseed group (80.0%; 72/90) followed by salmate (77.01; 67/87) and control groups (53.85%; 49/91). In addition, the number of services per conception and open days were approximately similar among groups.

### Blood biochemistry profiles

The blood biochemistry indices showed that TP (g/dl) were the highest (6.72;  $P=0.088$ ) in extruded flaxseed group compared to salmate (6.66) and control (6.70) ones. Simultaneously, globulin 2.89 vs. 2.75 and 2.82 g/dl), BUN (16.02 vs. 15.75 and 15.20 mg/dl),  $\beta$ -HBA (0.56 vs. 0.48 and 0.46 mmol/ml), cholesterol (200.18 vs. 170.66 and 152.75 mg/dl), acetate (0.66 vs. 0.64 and 0.47 nmol/ml), T4 (2.36 vs. 1.98 and 2.20 ng/dl), IGF (5.83 vs. 4.40 and 3.82 ng/ml), and insulin (0.65 vs. 0.54 and 0.410 ng/ml) values were followed the same trends. On the other hand, NEFA (mmol/l) were the lowest (0.24;  $P<0.0001$ ) in extruded flaxseed group compared to salmate (0.38) and control (0.40) ones as glucose (58.54 vs. 59.5 and 63.72 mg/dl), cortisol (14.07 vs. 22.40 and 20.20 ng/ml) values.

## DISCUSSION

Omega-3 and omega-6 families should be supplied in the diets because mammalian species cannot synthesize those fatty acids in their bodies. In addition, the supplied feed additives must be safe for body health and wellbeing of pregnant and lactating animals to support their ovarian follicles' development, milk production and composition (Al-Mufarji *et al.*, 2023; Al-Masruri *et al.*, 2022). The relationships among nutrition, reproductive performances and metabolic body health conditions have been explored in several studies (Moallem, 2018; Ali *et al.*, 2021; Al-Mufarji *et al.*, 2022, 2023; Mohammed and Al-Suwaiegh, 2023; Di Meo *et al.*, 2023). The current trial is long experiment conducted in commercial lactation farm to restore the negative effects of peripartum and postpartum periods in lactating Holstein cows. We present here part of results concerning reproductive traits and the related plasma metabolites and hormones (Tables I, II). The beneficial effects of omega-3 fatty acid on productive and reproductive performances and body health status were confirmed earlier in several short studies, which were differed in diet formulation and experimental conditions (Kra *et al.*, 2021; Di Meo *et al.*, 2023). The improvement in

reproductive performances and body health conditions in the current was highest in extruded flaxseed (7.0%) group if compared to salmate (25g/h/d) and control groups.

The results of supplementing cows with extruded flaxseed, the most widely available botanical source of n-3 FA, and salmate (dried fish oil) showed notable improvement in reproductive performance concerning earlier restoration of ovarian activities and higher conception rates. The FAs of extruded flaxseed and salmate might affect the ovarian structures development and quality, fertilization rate and further embryonic development (Petit, 2002; Ambrose *et al.*, 2006; Moallem, 2018). Santos *et al.* (2008) and Cerri *et al.* (2009) concluded that increased intake of polyunsaturated fatty acids (PUFAs) might affect the fatty acid composition of reproductive tissues and consequently improve fertilization rate and embryonic development.

It has been found that cows supplemented with encapsulated flaxseed at 3.8% of DM had higher numbers of 2- to 5-mm follicles on d 5 and 9 of the estrous cycle (Zachut *et al.*, 2010). In addition, cows supplemented with extruded flaxseed from 3 weeks pre-partum to 100 days postpartum had longer durations of estrous behavior and longer estrogen surge (Zachut *et al.*, 2011). Robinson *et al.* (2002) found higher plasma E2 concentration in cows fed  $\alpha$ -linolenic acid than in those fed linoleic acid. Holstein cows supplemented with rolled flaxseed and rolled sunflower for 32 d after insemination had larger ovulatory follicles and reduced pregnancy losses (9.8% vs. 27.3%) (Ambrose *et al.*, 2006).

Beyond ovarian follicle development, Petit and Benchaar (2007) found a higher conception rate at first artificial insemination in cows supplemented with whole flaxseed (54.30%) than those fed with micronized soybeans (40.0%), which might be attributed to larger well-developed corpora lutea as found in cows fed diets supplemented with fish oil or line seed (Petit, 2002). In a large-scale study, 4.0-5.0% extruded flaxseed diet resulted in fewer days from first artificial insemination to conception in addition to fewer open days (Moallem, 2018). In addition, cows fed 2.7-3.2% of DM fishmeal from 24 days pre-partum to 109 postpartum had higher pregnancy rate (41.3 vs. 31.9%). Petit and Twagiramungu (2006) found that cows fed whole flaxseed to 120 d postpartum showed no embryo mortality. Feeding algae increased resumption of estrous at day 58 postpartum and pregnancy at first artificial insemination, and increased pregnancy per artificial insemination, which reduced days to pregnancy by 22 d compared with control cows (Sinedino *et al.*, 2017). Collectively, the improvement in ovarian follicles and corpora lutea, embryo mortality, blood metabolites and hormones could explain higher reproductive performances in both extruded flaxseed and salmate groups.

The effects of supplementation extruded flaxseed (7.0%) and salmate (25 g/head/day) to lactating cows from three weeks pre-partum to 160 days postpartum on blood metabolites and hormone values are presented in [Tables II](#). It was observed that the values of blood metabolites and hormones in extruded flaxseed, salmate and control group were within the normal range ([Krogstad and Bradford, 2023](#)). Of note, in the present trial, values of total protein ( $P = 0.088$ ) and globulin ( $P = 0.053$ ) were increased in extruded flaxseed group compared to salmate and control groups due to the anti-inflammatory properties of omega-3 fatty acids in flaxseed ([Kra et al., 2021](#)). Blood urea nitrogen ( $P = 0.0016$ ) were increased in extruded flaxseed and salmate groups compared to control one. This is attributed to changes in feed digestibility and rumen fermentation (data not shown).

Extruded flaxseed diet resulted in lower ( $P < 0.0001$ ) concentration of plasma NEFA and higher concentration of  $\beta$ -HBA compared to salmate and control groups. Conjugated linoleic acid has been shown to decrease NEFA mobilization from body fat stores leading to lower circulating NEFA values ([Zhu et al., 2023](#)). In addition, the alteration of rumen fermentation patterns might lead to increase  $\beta$ HBA production as an alternative energy source, especially during periods of NEB. The values of glucose and TG were decreased ( $P < 0.0001$ ) in extruded flaxseed and salmate groups compared to control as a result of increasing milk production in those groups in addition to changes in milk composition and fatty acids profiles ([Moallem et al., 2020](#); [Beauregard et al., 2023](#)). Besides, cholesterol, HDL and acetate values were higher in in extruded flaxseed and salmate groups compared to control as in other studies ([Dail et al., 2011](#); [Utama et al., 2018](#)) due to the fat contents in flaxseed and salmate diets. In the regard of hormonal changes due to flaxseed and salmate diet; insulin, T4 and IGF-1 values were increased flaxseed group ( $P < 0.0001$ ) whereas cortisol values were decreased. Such hormonal changes are consistent with other changes in plasma metabolites as lower glucose concentration and increased milk production in addition to alleviation of stress.

## CONCLUSION

The results of extruded flaxseed (7.0%) or salmate (25 g/head/day) inclusion in diets of commercial lactating Holstein farm during transitional period till six months of lactation show notable positive effects on acceleration of ovarian activities in addition to significant increase of conception rate. Furthermore, the plasma metabolites and hormones values were within the normal ranges supporting body health and production.

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### IRB approval

The approval of the study was granted by the Ethical Research Committee of King Faisal University, Saudi Arabia.

### Ethical approval

Animals care in the current trial was approved of the scientific research deanship ethical standards of King Faisal University (Ref. No. KFU-REC).

### Statement of conflict of interest

The authors have declared no conflict of interest.

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