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Effect of Dietary Source of Omega-3 Fatty Acids on Milk Production, Fatty Acid Profiles and IGF-1 of Lactating Dairy Cows in Arid Subtropics

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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
Flaxseed, Salmate, Milk, Fat, Fatty acids, IGF-1

ABSTRACT

Dietary omega-3 fatty acids are a type of polyunsaturated fat known to improve production and body health in animals and human as well. The current on-farm trial was to evaluate the effects of omega-3 fatty acids inclusion in the diets on milk production and fatty acid profiles and insulin growth factors-1 values in lactating dairy cattle. Three hundred Holstein lactating cows in a commercial farm were assorted to a control group fed basal control diet and two treated groups fed diets containing extruded flaxseed (7.0%) and salmate (25 g/head/day). The basal control, extruded flaxseed and salmate diets were formulated to be isonitrogenous and isoenergetic diets. The diets were given to each group from three weeks pre-parturition to week 23 of lactation. Feed intake, milk production and composition and fatty acid profiles were recorded during the study. Fat and energy corrected milk were calculated. Feed intake did not differ among groups. Flaxseed significantly ($P<0.05$) increased milk yield, fat corrected milk and energy corrected milk if compared to other groups. Milk fat (%) decreased significantly ($P<0.05$), while saturated short-chain (C12:C15) and long-chain (C17:C20) fatty acids were higher ($P<0.05$) in Salmate and extruded flaxseed groups. Moreover, the ratio of omega-6/omega-3 was the lowest in flaxseed group (3.76) compared to control (8.06) and salmate (8.31) groups. In conclusion, flaxseed diet improved ($P < 0.05$) milk production efficiency (kg milk/kg feed) and fatty acid profile compared to salmate and control diets.

INTRODUCTION

Dietary supplements to ruminant animals affect both productive and reproductive performances (Mohammed and Al-Suwaiegh, 2023). The global demand for an adequate meat and milk is predicted to increase by 35.0% by 2030 due to increase demand. Saudi Arabia, as a result, has encouraged the development in meat and milk production to overcome the increasing demand (Al-Suwaiegh *et al*., 2022). Therefore, several strategies have been explored a worldwide including dietary supplements that can improve meat and milk production.

Mammalian species can synthesize fatty acids except omega-3 and omega-6 families, which need to be given in the diet. Omega-3 fatty acids are a type of polyunsaturated fat (PUFs) found in fish oil. Supplementation of essential PUFs to ruminant animals are faced with lipolysis and bio-hydrogenation in the rumen and little are available for absorption in addition to their toxic effect on rumen microbes. Therefore, fat manipulation is required to ensure reaching unsaturated FAs to small intestine for absorption. This can be achieved through feed ingredients, changing the environment of rumen, shifting rumen bypass (Ibrahim and Hassen, 2023; Wei *et al*., 2023).

Omega-3 fatty acids particularly obtained from flaxseed and fish oil are found essential for animals and human health (Karageorgou *et al*., 2023; Elbarbary *et al*., 2023; Dere-Yelken *et al*., 2024). Some studies have

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been explored the effects of omega-3 fatty acids on milk production and composition. The results might be variable due to experimental conditions, animal species, the doses and flaxseed processing (Leduc *et al*., 2017; Meignan *et al*., 2017). Studies of omega-3 fatty acids on milk production and composition have shown no significant changes, while others reported slight increases or decreases depending on the type and amount of omega-3 fatty acids supplemented (Moallem *et al*., 2020; Beauregard *et al*., 2023). Flaxseed supplementation, particularly to high-yielding cows, has shown some potential to increase milk production. In addition, fish oil supplementation may have a a variable effect depending on the dose, where moderate doses may slightly increase milk production, while higher doses may suppress it due to it is potential negative effect on rumen function (Swanepoel and Robinson, 2020; Ferlay and Chilliard, 2020).

Fat content and fatty acid profiles of milk are highly correlated with quality, processing ability, sensory properties and body health. Regarding to effects of omega-3 fatty acids on milk composition, it has been found that omega-3 supplementation significantly altered the fatty acid profile of milk, increasing the proportion of omega-3 fatty acids and decreasing the ratio of omega-6 to omega-3 fatty acids (Petit, 2003; Ruiz-González *et al*., 2022). This shift in fatty acid profile can potentially improve the nutritional value of milk. Other milk components including lactose and minerals are generally not affected by omega-3 supplementation. Additionally, due to the direct and indirect roles of IGF-1 on milk production including mammary gland development, milk synthesis, nutrient uptake, hormonal regulation and energy balance (Meyer *et al*., 2017), the changes of IGF-1 were explored during the study. Therefore, this study was designed to investigate sources of omega-3 fatty acids including extruded flaxseed and salmate, on IGF-1, efficiency of milk production and fatty acid profile of lactating dairy cattle in arid subtropics.

MATERIALS AND METHODS

Animals management and diets

The current study was carried out in a dairy farm located in Riyadh of KSA from June to December 2023. Three hundred lactating Holstein cows were used for the study. The cows had 582±21 kg of body weight and 3.35±0.10 of body condition score.

The average ambient temperature ranged between 31 to 48 degrees Celsius and relative humidity ranged between 8 to 94 in Riyadh region of KSA. It is important to indicate that all cows were kept under evaporative cooling system, which works during stressful condition. The evaporative cooling system in the dairy farm starts to work automatically when the temperature inside the barns reaches 21°C. The fans will start first then cooling system by water spray when the temperature increases to 23 °C. Consequently, the average temperature was recorded as 25°C to 28°C by using that system. Animals were placed in an open lot and were divided equally into three groups based on previous average milk yield and parity: Second lactation (L2= 37 cows), third lactation (L3= 35 cows), fourth lactation (L4=16 cows) fifth lactation (L5=12 cows), and the average milk yield includes control (32.27kg), dry protected fish oil (Salmate) (33.70kg) and Flaxseed/linseed (28.99kg). The cows were randomly distributed through complete randomized design for investigating the effects of extruded flaxseed (7.0%) and salmate (25g/head/day) compared to basal control diet. The period of experiment lasted approximately six months. The control, extruded flaxseed and salmate diets were formulated to provide the nutrient requirement of lactating dairy cows according to body weight (580.0 kg) and body gain (0.2 kg day^{-1}), milk production (17 kg day^{-1}) and milk fat (3.5%) as recommended by NRC (2001). The cows were given *ad libitum* mineral mixture and fresh water. The rations of extruded flaxseed and salmate were prepared weekly to avoid lipid peroxidation. Sufficient amount of control, extruded flaxseed and salmate rations was offered to the lactating cows as a total mixed ration to allow for a 10.0% ort. Feed intake was recorded as the difference between the daily quantity of ration that was offered and its respective ort.

Extruded flaxseed and salmate supplements

Extruded flaxseed ingredient includes 85 g/kg flaxseed and 15 g/kg wheat bran obtained from ARASCO (ARASCO R and D Guidelines, Riyadh, KSA). The Chemical composition of extruded flaxseed is 44.0 fat, 28.0% fiber, 21.0% protein, 4% ash, 6% carbohydrates. The extruded flaxseed fat contains 47.0% omega-3 fatty acid (α -linolenic fatty acid, ALA C18:3 n-3), and 15.0 % omega-6 fatty acid (mostly linoleic acid, LA 18:2 n-6). Salmate is dried fish oil and natural protected source of polyunsaturated fatty acid. The salmate contains about 31.0% omega- 3 and 5.0% Omega-6 fatty acids.

Meteorological condition

In the commercial dairy farm, there is a monitoring station for recording the temperatures and humidity daily during the entire experimental period. Temperature-humidity index (THI) was calculated to quantify the degree of heat stress on cows using the following formula (West, 2003), THI = td - (0.5 x RH) (td - 58), where td is the dry bulb temperature in ˚F, and RH is the relative humidity as a fraction of the unit (Fig. 1).

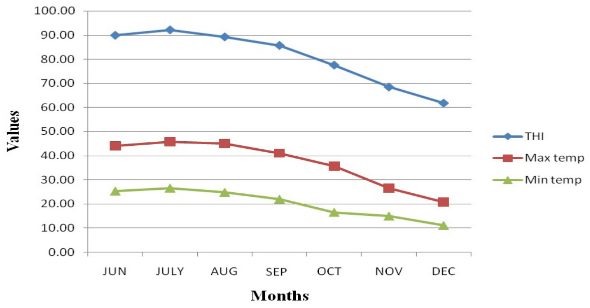

Fig. 1. Maximum, minimum temperatures and temperature-humidity index (THI) during the experimental period.

Body condition score

Body condition score (BCS) using a five-point scale (where1 = emaciated and 5 = fat according to Wildman *et al*. (1982) were recorded at the beginning of the trial, after two months and at the end of the trial.

Milk sample collection and chemical analysis

Milk production and other information of control, extruded flaxseed, salmate groups were recorded electronically in milking parlor during the experimental period. Milk samples (50.0 mL) were collected from the control and experimental groups and stored in tubes for further chemical analysis. Milk samples of control and experimental groups, extruded flaxseed and salmate were analyzed for determination of casein, fat, protein, total solids (TS), solids non-fat (SNF), lactose, milk urea nitrogen. Fat corrected milk (3.5% fat) and energy corrected milk (3.5% fat and 3.2% protein) were calculated, respectively, according to formulas of Nordlund (1987) and Bernard (1997). Fatty acid contents were calculated as described by Glasser *et al*. (2007). In addition, the collected milk samples were stored (-20°C) without preservative to determine the FAs profiles using gas chromatography (Cruz-Hernandez *et al*., 2007).

Insulin-growth factor-1

Blood samples were collected biweekly and plasma samples were obtained and stored (-20°C). Insulin-growth factor-1(IGF-1) was determined by inhibition immunoassay technique (ELISA), using commercial kit (Ycusabio Company, Japan).

Statistical analysis

Data of feed intake, milk production, fatty acid profiles and IGF-1 values were statistically analyzed by generalized linear model procedure of SAS (2008) using the following model; $Y_{ijkl} = \mu + T_i + E_{ij}$; where Y_{ij}, the observation; M, the overall mean; E_{ij}, standard error. Duncan's Multiple Range Test (Duncans, 1955) was used to compare the means of the control and treated groups.

RESULTS

Feed intake (kg) and milk yield (kg/day)

Table I shows that dry matter intake increased (P > 0.05) in salmate groups when compared to the flaxseed and control groups during pre-partum period. During postpartum period, the dry matter intake showed a decreasing trend (P > 0.05) in salmate and extruded flaxseed groups when compared to the control group. Tables II presents the milk yield of lactating cows fed with the experimental diets (control, salmate and extruded flaxseed) over twenty-three weeks. Average milk yield was 46.87, 42.24 and 41.24 kg/d of extruded flaxseed, salmate and control groups, respectively. Extruded flaxseed and salmate diets increased milk yield by 13.65%, and 2.4%, respectively, compared to control diet. Extruded flaxseed significantly (P<0.05) increased energy corrected milk. Fat corrected milk was not affected by feeding flaxseed compared to the control. Highest feed conversion ratio (1.78 kg milk/kg feed) was obtained in extruded flaxseed group followed by Salmate (1.58 kg milk/kg feed (and control diet (1.52 kg milk/kg feed. The difference in body condition score among groups was not significantly (P>0.05) differed.

Table I. Effect of salmate and extruded flaxseed diets on feed intake (dry matter basis) of lactating Holstein cows.

Fatty acids	Control	Treatments	
		Salmate	Flaxseed
Prepartum, 21 days	11.24±0.81	13.23±0.81	11.95±0.81
Postpartum, 14 days	19.02±1.243	18.76±1.24	17.13±1.24
Postpartum,14 to 160 days	26.98±0.71	26.65±0.71	26.23±0.71

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.

Saturated short-chain fatty acids

Table III shows saturated short-chain fatty acids of lactating Holstein cows. The value of C4:0 was significantly (P < 0.05) lower (0.64) in flaxseed group when compared to salmate (0.77) and control (0.74) groups. There was no significant difference in FAs values among all groups (C6:0 to C11:0). The value of C12:0 was significantly (P<0.05) higher in flaxseed group (2.62) compared to salmate (2.37) and control (2.33) groups. The values of C14:0 and C15:0

were significantly higher in flaxseed group compared to salmate and control treatments.

Table II. Effect of salmate and extruded flaxseed diets on milk production and efficiency of lactating Holstein cows.

Fatty acids	Control	Treatments	
		Salmate	Flaxseed
Milk yield (kg/d)	41.24±0.26^{c}	42.24±0.26^{b}	46.87±0.26^{a}
Fat (%)	3.68±0.05^{a}	3.38±0.05^{b}	3.04±0.06^{c}
Fat corrected milk (kg/d)	42.72±0.51^{a}	41.93±0.52^{b}	43.77±0.55^{a}
Energy corrected milk (kg/d)	41.42±0.44^{b}	40.65±0.45^{b}	43.59±0.47^{a}
Production efficiency, (kg milk/kg feed)	1.52±0.81^{b}	1.58±0.81^{b}	1.78±0.81^{a}
Body condition score	3.41±0.01	3.18±0.01	3.48±0.01

$^{a, b, and c}$; values in the same row with different superscripts differ significantly (P < 0.05). For details of treatments, see Table I.

Table III. Effect of salmate and extruded flaxseed diets on saturated short-chain and long chain fatty acids of lactating Holstein cows.

Fatty acids	Control	Treatments	
		Salmate	Flaxseed
Short chain fatty acid			
C4:0	0.74±0.02^{a}	0.77±0.02^{a}	0.64±0.02^{b}
C6:0	0.60±0.01	0.58±0.01	0.58±0.01
C8:0	0.57±0.009	0.57±0.009	0.59±0.01
C9:0	0.04±0.01	0.03±0.01	0.03±0.01
C10:0	1.63±0.14	1.88±0.14	1.84±0.16
C11:0	0.06±0.01	0.04±0.01	0.04±0.01
C12:0	2.33±0.03^{b}	2.37±0.03^{b}	2.62±0.04^{a}
C13:0	0.09±0.003^{a}	0.07±0.003^{b}	0.09±0.004^{a}
C14:0	9.38±0.10^{b}	9.44±0.10^{b}	10.45±0.11^{a}
C15:0	0.88±0.01^{b}	0.83±0.01^{c}	0.94±0.01^{a}
Long change fatty acid			
C16:0	45.34±0.27^{a}	46.03±0.28^{a}	38.20±0.30^{b}
C17:0	0.41±0.005^{b}	0.39±0.005^{c}	0.44±0.006^{a}
C20:0	0.10±0.008^{b}	0.09±0.008^{b}	0.13±0.009^{a}
C22:0	0.16±0.007	0.14±0.007	0.15±0.007
C23:0	0.13±0.009	0.12±0.01	0.13±0.01
C24:0	0.13±0.01	0.14±0.01	0.13±0.01

For details of treatments, see Table I.

Saturated long-chain fatty acids

The data of saturated long-chain fatty acids values is shown in Table III. The value of C16:0 was significantly higher in salmate (46.03) and control (45.34) groups compared to flaxseed group (38.20). The value of C17:0 was significantly higher (P<0.05) in flaxseed group (0.44) versus control (0.41) and Salmate (0.39) groups. The value of C20:0 was significantly higher (P<0.05) in flaxseed diet (0.13) compared to control (0.10) and salmate (0.09) groups. There was no significant difference in values of fatty acids (C22:0, C23:0 and C24:0) among all groups.

Monounsaturated fatty acids

The data of monounsaturated fatty acids values is shown in Table IV. The values of C10:1 and C20:1 was not differed among groups. The values of C14:1 was significantly (P<0.05) higher in the flaxseed group compared to salmate and control group. On the other hand, the value of C16:1 was significantly higher in salmate and control groups when compared to flaxseed group. The values of C18:1 and oleic acid was significantly higher in flaxseed group when compared to the salmate and control groups.

Table IV. Effect of salmate and extruded flaxseed diets on monounsaturated and polyunsaturated fatty acids of lactating Holstein cows.

Fatty acids	Control	Treatments	
		Salmate	Flaxseed
Monounsaturated fatty acids			
C10:1	0.10±0.007	0.11±0.007	0.11±0.007
C14:1	0.79±0.02^{b}	0.78±0.02^{b}	0.97±0.02^{a}
C16:1	1.56±0.02^{a}	1.57±0.02^{a}	1.49±0.03^{b}
C18:1	0.70±0.04^{c}	0.82±0.04^{b}	1.10±0.04^{a}
C18:1 (Oleic)	20.19±0.20^{b}	20.16±0.20^{b}	22.75±0.22^{a}
C20:1	0.35±0.04	0.32±0.04	0.43±0.04
Polyunsaturated fatty acids			
C18:2 (Omega-6)	2.66±0.03^{b}	2.66±0.03^{b}	2.86±0.03^{a}
C18:3 (Omega-3)	0.33±0.01^{b}	0.32±0.01^{b}	0.76±0.01^{a}
C20:4	0.16±0.006	0.15±0.006	0.14±0.006
C20:5	0.12±0.009	0.12±0.009	0.12±0.009
C20:6	0.17±0.01^{b}	0.19±0.01^{a}	0.15±0.01^{c}
Omega-6:Omega-3	8.06	8.31	3.76

For details of treatments, see Table I.

Polyunsaturated fatty acids

The data of polyunsaturated fatty acids values is shown in Table IV. The value of C18:2 (Omega-6) was significantly (P< 0.05) higher in flaxseed group compared to salmate and control groups. The same trend was found in value of C18:3 (Omega-3). More importantly, the omega-6/omega-3 ratio decreased by supplementation of flaxseed (3.76) compared to control (8.06) and salmate (8.31) groups.

Insulin growth factor-1

The data of insulin growth factor-1 (IGF-1) values is shown in Table V. The highest values of IGF-1 (P < 0.05) was found in flaxseed group followed by salmate and control groups, respectively. It seems that the values of IGF-1 in the groups follow the milk production curve in cattle, where the values of IGF-1increased till week 6 and decreased thereafter in all groups.

Table V. Effect of salmate and extruded flaxseed diets on insulin growth factors-1 of lactating Holstein cows.

Weeks	Insulin growth factor-1		
	Control	Salmate	Flaxseed
Week 0	3.63 ± 0.06^{b}	3.45 ± 0.09^{b}	5.95 ± 0.24^{a}
Week 2	4.45 ± 0.22^{b}	4.08 ± 0.09^{b}	6.53 ± 6.58^{a}
Week 4	4.68 ± 0.14^{b}	4.37 ± 0.07^{b}	6.58 ± 0.11^{a}
Week 6	4.42 ± 0.11^{c}	5.15 ± 0.09^{b}	7.36 ± 0.22^{a}
Week 8	4.34 ± 0.06^{b}	5.14 ± 0.09^{b}	6.57 ± 0.16^{a}
Week 10	4.23 ± 0.06^{b}	4.82 ± 0.11^{b}	6.19 ± 0.13^{a}
Week 12	3.89 ± 0.09^{b}	4.69 ± 0.11^{b}	6.13 ± 0.16^{a}
Week 14	3.87 ± 0.06^{b}	4.68 ± 0.04^{b}	5.85 ± 0.08^{a}
Week 16	3.82 ± 0.08^{b}	4.68 ± 0.03^{b}	5.88 ± 0.16^{a}
Week 18	3.61 ± 0.06^{c}	4.58 ± 0.09^{b}	5.74 ± 0.11^{a}
Week 20	3.57 ± 0.07^{c}	4.49 ± 0.07^{b}	5.72 ± 9.04^{a}
Week 22	2.99 ± 0.04^{c}	4.48 ± 0.09^{b}	5.50 ± 0.15^{a}
Week 24	2.93 ± 0.06^{c}	4.39 ± 0.09^{a}	5.28 ± 0.09^{a}
Week 26	2.81 ± 0.04^{b}	4.11 ± 0.10^{b}	4.43 ± 0.06^{a}
Overall	3.85 ± 0.11^{b}	3.84 ± 0.12^{b}	5.88 ± 0.16^{a}

For details of treatments, see Table I.

DISCUSSION

The effects of supplementation extruded flaxseed (7.0%) and salmate (25 g/head/day) to Frisian cows from three weeks pre-partum to 160 days postpartum on milk production and efficiency, fatty acid profiles and insulin growth factors-1 (IGF-1) is presented in Tables I-V. The results indicated that feed intake was none significantly (P > 0.05) decreased in salmate and extruded flaxseed groups. Values of milk yield (kg), fat (%), fatty acid profiles and IGF-1 were improved upon feeding extruded flaxseed diet.

Omega-3 and omega-6 families should be supplied in the diets because mammalian species cannot synthesize them. Furthermore, the problem with supplementation of essential fatty acids (FAs) to ruminant animals is occurrence of lipolysis and bio-hydrogenation of FAs in the rumen. Very little unsaturated FAs are available for absorption and the toxic effect of unsaturated FAs to rumen microbes is indicated (Vargas *et al*., 2020). Therefore, pathway of rumen bio-hydrogenation requires fat manipulation to ensure reaching dietary unsaturated FAs to small intestine in the form of conjugated linoleic acid (CLA). Several studies were previously evaluated the effects of fat supplementation on milk production and composition (Sadeghi *et al*., 2019; Al-Mufarji *et al*., 2023). However, caution is taken of dietary fat supplementation because of the significant decreases of feed intake (Maigaard *et al*., 2023).

The feed intake (P > 0.05) in salmate and extruded flaxseed groups were slightly decrease during postpartum period compared to control one, which might be attributed to generation of feedback satiety signals that prevent further feed intake over feeding large amounts of fats (Palmquist and Jenkins, 1980). Extruded flaxseed and salmate diets resulted in greater milk yield compared to control diet. Extruded flaxseed and salmate diets increased milk yield by 13.65%, and 2.4%, respectively. In addition, milk fat (%) was significantly reduced in extruded flaxseed and salmate groups compared to control groups (3.04, 3.38 vs. 3.68). This is consistent with supplementing 200 g/d of fish oil, which resulted in a 26.0% reduction in milk fat compared to 200 g/d of olive oil (Mattos *et al*., 2004). When energy corrected milk was calculated, extruded flaxseed diet significantly (P<0.05) increased energy corrected milk compared to other groups. In this regard, the addition of 0.5-0.7 kg diet/d per cow from 21 days prepartum to 7 months postpartum has a potential effect on the milk yield of dairy cows (Moallem *et al*., 2020; Beauregard *et al*., 2023). The boost in milk production of extruded flaxseed diets could be highly related to their effects on comparable dry-matter intake (Table I), improved feed digestibility, increased energy intake and metabolism, improved rumen function (Moats *et al*., 2018) and enhanced hormonal activity including insulin growth factors-1 (Table V). In addition, FFAs seemed to cause a state of insulin resistance, increase the amount of glucose for synthesis of lactose and consequently for milk production (Andersen *et al*., 2008). Milk production efficiency (kg milk/kg feed) was significantly improved over feeding extruded flaxseed diet when compared to salmate and control diets (Table I). Of note, in the present trial, changes were observed in not only the milk yield by the addition of extruded flaxseed to the diets of lactating cows, but in the fat and energy corrected milk also as indicated in other studies (Moallem *et al*., 2020; Beauregard *et al*., 2023). Beauregard *et al*. (2023) estimated that CH4 intensity was reduced by 1.3 g/L of milk (9.2%) in herds receiving extruded flaxseed. Moreover, the values of IGF-1 indicated the highest in flaxseed group followed by salmate and control groups, respectively. It seems that the values of IGF-1 in the groups follow the milk production curve in cattle, where the values of IGF-1 increased till week 6 and decreased thereafter in all groups. This is consistent with the direct and indirect roles of IGF-1 on milk production through mammary gland development, milk synthesis, nutrient uptake, hormonal regulation and energy balance (Meyer *et al*., 2017).

CONCLUSION

The results of the current trial show that the extruded flaxseed (7.0%) or salmate (25 g/head/day) in diets of commercial lactating Holstein farm did not show any notable negative effects on feed intake. Additionally, milk production, fat and energy corrected milk, milk efficiency (kg milk/kg feed) were significantly improved due to extruded flaxseed feeding. The positive effects extended to milk composition through the ration of omega-6: Omega-3 ratio. Simultaneous improvement was confirmed in IGF-1 through the period of study.

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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 statement

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

Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Al-Mufarji, A., Al-Suwaiegh, S. and Mohammed, A.A., 2023. Influence of organic *Moringa oleifera* leaves supplemented during gestation and lactation periods: Modulation of production efficiency, blood and metabolic parameters of ewes and lambs in subtropics. *Adv. Anim. Vet. Sci.*, **7**: 385-393. https://doi.org/10.17582/journal.aavs/2023/11.3.385.393

Al-Suwaiegh, S.B., Almotham, A.M., Alyousef, Y.M., Mansour, A.T. and Al-Sagheer, A.A., 2022. Influence of functional feed supplements on the milk production efficiency, feed utilization, blood metabolites, and health of Holstein cows during mid-lactation. *Sustainability,* **14**: 8444. https://doi.org/10.3390/su14148444

Andersen, J.B., Ridder, C. and Larsen, T., 2008. Priming the cow for mobilization in the periparturient period: Effects of supplementing the dry cow with saturated fat or linseed. *J. Dairy Sci.,* **91**: 1029-1043. https://doi.org/10.3168/jds.2007-0437

Beauregard, A., Dallaire, M.P., Gervais, R. and Chouinard, P.Y., 2023. Lactational performance of cows fed extruded flaxseed in commercial dairy herds. *Anim. Open Space,* **2**: 100043. https://doi.org/10.1016/j.anopes.2023.100043

Bernard, J.K., 1997. Milk production and composition responses to the source of protein supplements in diets containing wheat middlings. *J. Dairy Sci.,* **80**: 938-942. https://doi.org/10.3168/jds.S0022-0302(97)76017-X

Cruz-Hernandez, C., Kramer, J.K.G., Kennelly, J.J., Glimm, D.R., Sorensen, B.M., Okine, E.K., Goonewardene, L.A. and Weselake, R.J., 2007. Evaluating the conjugated linoleic acid and trans 18:1 isomers in milk fat of dairy cows fed increasing amounts of sunflower oil and a constant level of fish oil. *J. Dairy Sci.,* **90**: 3786-3801. https://doi.org/10.3168/jds.2006-698

Dere Yelken H., Elci, M.P., Turker, P.F. and Demirkaya, S., 2024. Omega fatty acid ratios and neurodegeneration in a healthy environment. *Prostagland. Other Lipid Mediat*., **170**: 106799. https://doi.org/10.1016/j.prostaglandins.2023.106799

Duncan, D.B., 1955. Multiple range and multiple Ftest. *Biometrics,* **11**: 1. https://doi.org/10.2307/3001478

Elbarbary, N.S., Ismail, E.A. and Mohamed, S.A., 2023. Omega-3 fatty acids supplementation improves early-stage diabetic nephropathy and subclinical atherosclerosis in pediatric patients with type 1 diabetes: A randomized controlled trial. *Clin. Nutr.,* **42**: 2372-2380. https://doi.org/10.1016/j.

clnu.2023.10.007
Ferlay, A. and Chilliard, Y., 2020. Effect of linseed, sunflower, or fish oil added to hay-, or corn silage-based diets on milk fat yield and trans-C18:1 and conjugated linoleic fatty acid content in bovine milk fat. *Livest. Sci.*, **235**: 104005. https://doi.org/10.1016/j.livsci.2020.104005
Glasser, F., Doreau, M., Ferlay, A. and Chilliard, Y., 2007. Technical note: Estimation of milk fatty acid yield from milk fat data. *J. Dairy Sci.*, **90**: 2302–2304. https://doi.org/10.3168/jds.2006-870
Ibrahim, S.L., and Hassen, A., 2022. Effect of non-encapsulated and encapsulated mimosa (*Acacia mearnsii*) tannins on growth performance, nutrient digestibility, methane and rumen fermentation of South African mutton Merino ram lambs. *Anim. Feed Sci. Technol.*, **294**: 115502. https://doi.org/10.1016/j.anifeedsci.2022.115502
Karageorgou, D., Rova, U., Christakopoulos, P., Katapodis, P., Matsakas, L. and Patel, A., 2023. Benefits of supplementation with microbial omega-3 fatty acids on human health and the current market scenario for fish-free omega-3 fatty acid. *Trends Fd. Sci. Technol.*, **136**: 169-180. https://doi.org/10.1016/j.tifs.2023.04.018
Leduc, M., Létourneau-Montminy, M.P., Gervais, R. and Chouinard, P.Y., 2017. Effect of dietary flax seed and oil on milk yield, gross composition, and fatty acid profile in dairy cows: A meta-analysis and meta-regression. *J. Dairy Sci.*, **100**: 8906-8927. https://doi.org/10.3168/jds.2017-12637
Maigaard, M., Weisbjerg, M.R., Johansen, M., Walker, N., Ohlsson, C. and Lund, P., 2023. Effects of dietary fat, nitrate, and 3-NOP and their combinations on methane emission, feed intake and milk production in dairy cows. *J. Dairy Sci.*, **2023**: 23420. https://doi.org/10.3168/jds.2023-23420
Mattos, R., Staples, C.R., Arteche, A., Wiltbank, M.C., Diaz, F.J., Jenkins, T.C. and Thatcher, W.W., 2004. The effects of feeding fish oil on uterine secretion of PGF2α, milk composition, and metabolic status of periparturient Holstein cows. *J. Dairy Sci.*, **87**: 921-932. https://doi.org/10.3168/jds.S0022-0302(04)73236-1
Meignan, T., Lechartier, C., Chesneau, G. and Bareille, N., 2017. Effects of feeding extruded linseed on production performance and milk fatty acid profile in dairy cows: A meta-analysis. *J. Dairy Sci.*, **100**: 4394-4408. https://doi.org/10.3168/jds.2016-11850
Meyer, Z., Höflich, C., Wirthgen, E., Olm, S., Hammon, H.M. and Hoeflich, A., 2017. Analysis of the IGF-system in milk from farm animals – Occurrence, regulation, and biomarker potential, *Growth Horm. IGF Res.*, **35**: 1-7. https://doi.org/10.1016/j.ghir.2017.05.004
Moallem, U., Lehrer, H., Livshits, L. and Zachut, M., 2020. The effects of omega-3 α-linolenic acid from flaxseed oil supplemented to high-yielding dairy cows on production, health, and fertility. *Livest. Sci.*, **242**: 104302. https://doi.org/10.1016/j.livsci.2020.104302
Moats J., Mutsvangwa, T., Refat, B. and Christensen, D.A. 2018. Evaluation of whole flaxseed and the use of tannin-containing fava beans as an alternative to peas in a co-extruded flaxseed product on ruminal fermentation, selected milk fatty acids, and production in dairy cows. *Prof. Anim. Scient.*, **34**: 435-446. https://doi.org/10.15232/pas.2018-01726
Mohammed, A.A. and Al-Suwaiegh, S., 2023. Impacts of *Nigella sativa* inclusion during gestation and lactation on ovarian follicle development, milk composition as well as the blood and metabolic profiles of Ardi goat in subtropics. *Agriculture*, **13**: 674. https://doi.org/10.3390/agriculture13030674
NRC, 2001. *Nutrient requirements of dairy cattle*, 7th Rev. ed. Nat. Acad. Press, Washington DC.
Nordlund, K., 1987. Adjusted corrected milk. *Bovine Prod.*, **19**: 87-89. https://doi.org/10.21423/aabppro19867572
Palmquist, D.L. and Jenkins, T.C., 1980. Fat in lactation rations. A review. *J. Dairy Sci.*, **63**: 1-14. https://doi.org/10.3168/jds.S0022-0302(80)82881-5
Petit, H.V., 2003. Digestion, milk production, milk composition, and blood composition of dairy cows fed formaldehyde treated flaxseed or sunflower seed. *J. Dairy Sci.*, **86**: 2637-2646. https://doi.org/10.3168/jds.S0022-0302(03)73859-4
Ruiz-González, A., Ramirez-Mella, M., Chouinard, P.Y., Gervais, R. and Rico, D.E., 2022. O159 Abomasally infused fish oil n-3 fatty acids increase milk production and reduce clinical signs of heat stress in dairy cows. *Anim. Sci. Proc.*, **13**: 440-441. https://doi.org/10.1016/j.anscip.2022.07.169
SAS, 2008. *SAS user's guide: Basics*. Statistical Analysis System Institute, Inc., Cary, NC, USA.
Sadeghi, M., Ghorbani, G.R., Ghasemi, E., Kargar, S., Leskinen, H., Bayat, A.R. and Ghaffari, M.H., 2019. Source of supplemental dietary fat interacts with relative proportion of forage source in Holstein dairy cows: Production responses, milk fat composition, and rumen fermentation. *Livest. Sci.*, **227**: 143-152. https://doi.org/10.1016/j.livsci.2019.07.016

Seleem M.S., Wu, Z.H., Xing, C.Q., Zhang, Y., Hanigan, M.D. and Bu, D.P., 2023. Impacts of rumen-encapsulated methionine and lysine supplementation and low dietary protein on nitrogen efficiency and lactation performance of dairy cows. *J. Dairy Sci.,* **2023**: 23404. https://doi.org/10.3168/jds.2023-23404

Swanepoel, N. and Robinson, P.H., 2020. Impacts of feeding a fish-oil based feed supplement through 160 days in milk on reproductive and productive performance, as well as the health, of multiparous early-lactation Holstein cows. *Anim. Feed Sci. Technol*., **268**: 114618. https://doi.org/10.1016/j.anifeedsci.2020.114618

Vargas, J.E., Andrés, S., Lorena López-Ferreras, L. and López, S., 2020. Effects of supplemental plant oils on rumen bacterial community profile and digesta fatty acid composition in a continuous culture system (RUSITEC). *Anaerobe*, **61**: 102143. https://doi.org/10.1016/j.anaerobe.2019.102143

Wei, X., Wu, H., Wang, Z., Zhu, J., Wang, W., Wang, J., Wang, Y. and Wang, C., 2023. Rumen-protected lysine supplementation improved amino acid balance, nitrogen utilization and altered hindgut microbiota of dairy cows. *Anim. Nutr.,* **15**: 320-331. https://doi.org/10.1016/j.aninu.2023.08.001

West, J.W., 2003. Effects of heat-stress on production in dairy cattle. *J. Dairy Sci.,* **86**: 2131-2144. https://doi.org/10.3168/jds.S0022-0302(03)73803-X

Wildman, E.E., Jones, G.M., Wagner, P.E., Boman, R.L., Troutt Jr, H.F. and Lesch, T.N., 1982. A dairy cow body condition scoring system and its relationship to selected production characteristics. *J. Dairy Sci.,* **65**: 495–501. https://doi.org/10.3168/jds.S0022-0302(82)82223-6