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Alterations of Productive and Reproductive Efficiencies in Dairy Cows by the Inclusion of Protected Fats and Full-Fat Soy in the Diet

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Abstract

This study aimed to explore the impacts of protected fat and full-fat soybean on the dry matter intake, milk production and composition, blood metabolites and reproductive traits of early lactating dairy cows. Eighteen dairy cows at early lactation were randomly assigned into two equal groups (G; n= 9/G). Cows were housed in semi-shaded open barn with free access to fresh water and salt blocks licks. Cows of MEGALAC group (control) were fed on the basal diet of a concentrate mixture containing 10% soybean and supplementation of 600 g of protected fat /cow/ day and green roughage was offered three times a day. Cows of Full-Fat Soy group were offered a diet in which the soybean meal was replaced by extruded full-fat soy (10%) without a supplement of MEGALAC and green roughage as above. Results revealed that mean value of dry matter intake, feed efficiency, milk SNF, lactose and total solids were higher in the MEGALAC group. MEGALAC diet tended to increase milk yield, fat corrected milk and energy corrected milk. Conversely, milk fat/ protein ratio was higher in Full- Fat Soy group, however Full- Fat Soy diet decreased ($P< 0.05$) capric acid (C10:0), but tended to increase oleic acid (C18:1). Serum omega-3 fatty acid was higher in the MEGALAC group, while the ratio of omega-6/ omega-3 was lower in the MEGALAC group than Full- Fat Soy group. Full- Fat Soy diet increased serum albumin and albumin/ globulin ratio, while decreased serum globulin and urea. Concentrations of both Non-Esterified Fatty Acids (NEFA) and β-Hydroxy Butyrate (BHB) were not different between groups. Mean pulse rate was higher in MEGALAC group. Moreover, rectal temperature ranges within the normal values in MEGALAC group, but not in Full- Fat Soy group. MEGALAC diet decreased rectal temperature and respiration rate but not significant in cows exposed to heat stress.

In conclusion, evidently, protected fat supplementation given to early lactating-dairy cows has shown positive impacts on their productive and reproductive traits.

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Introduction

The biological cycles of milk production and reproduction in dairies determine its profitability, thus making management decisions dynamic and time-dependent. Generally, high -yielding dairy cows are in a state of Negative Energy Balance (NEB) especially postpartum. This negative energy stems from the fact that the energy required for milk production and maintenance of body tissue function exceeds the amount of energy available from the diet [1]. Milk production peaks approximately six weeks post-calving, whereas feed intake peak occurs four weeks later or at ten weeks after calving. During this ten-week period, the cow is in an NEB [2,3], since the animal forcing mobilization of body fat reserves and skeletal muscle breakdown to provide sufficient energy requirements [4]. Accordingly, the main challenge in the nutrition of high-producing dairy cows is the provision of the proper amount of energy and protein in this critical period. For achieving this approach, there are several sources of energy that can be used in the diets of dairy cows to cover the energy requirements in the transition period such as grains and oilseeds [1]. However, excessive grains feeding increases dietary energy which might disturb rumen function (i.e. acidosis) and causes milk fat depression [5]. In addition, Santoshi et al., (2018) [6] reported that the combined supplementation of trheatisodium citrate (25gm) and vitaminE (1000 IU)/ animal/ day during the transition period improved body condition score and productivity of indigenous dairy cows. Feeding fat can increase energy density of the diet; however, feeding high amounts of unprotected fat can be toxic to microbial populations and exerts detrimental effects on rumen fermentation. Therefore, other energy sources such as protected fats, full-fat soy and glucogenic supplements have additional progress rather than increasing the energy density of the diet [7]. If the free carboxyl end of unsaturated fatty acids is protected from bacterial enzymatic attack in the rumen, the biohydrogenation cannot occur. Numerous studies have found that binding the unsaturated fatty acids with calcium salts protect these acids from biohydrogenation, and allow them to remain intact in the milk of ruminants in addition to protecting microbes from exerting toxic effects on unsaturated fatty acids [8,9].

Commercially, there are well-known products available in the markets such as MAGNAPAC and MEGALAC which are produced by reacting palm fatty acids distillate with calcium hydroxide to form calcium soap. This process protects the fats and allows it to largely bypass the rumen, reducing degradation, and allowing the fats to be efficiently utilized by the cow [10].

Several studies have described that feeding rumen-bypass fats to dairy cows in early lactation, increased fat-corrected milk yield [11], milk and fat-corrected milk yields [9,12], and milk fat percentage [13] without affecting the digestibility of other dietary nutrients. Full-fat soybean is one of the major oilseeds consisting of 17 to 20% oil on a dry matter basis. Full-fat soybeans as raw, roasted, extruded, rolled or ground may be successfully fed to dairy cows. The type of processing can affect the quality of the product, oil release in the rumen, and influence of nutrients utilization [14]. Instantly, grinding or rolling may increase utilization, depending on the total ration, and may make handling easier. However, grinding or rolling may increase the risk of rancidity for raw soybeans. On the other hand, extrusion has been shown to enhance the ruminal availability of soy oil; however, roasting may slow the rate of release of soy oil into the rumen [15]. Privé et al. (2010) [16] proposed that processing oilseeds with heat may have effects on ruminal lipid digestion which might be caused by a modification of the seed coat protection, a reduction of the amount of polyunsaturated fatty acids subjected to biohydrogenation, or the production of oxidation. Roasting soybeans decreases the ruminal degradability of the protein. For example, protein degradability in raw soybeans is about 72%, whereas protein degradability in roasted soybeans is about 50%. Extruded/expelled Soybean Meal (ESBM) is produced by mechanical friction creating a high temperature for a short time period. The temperature and the time spent at a given temperature directly affect the quality and nutritional value of the product. ESBM has the potential to be a source of high-quality protein and oil if processed correctly. After extrusion, soybeans (~18% fat) are expelled (pressed) to remove the oil [17]. ESBM (the final product) has about 7% fat compared to < 1% fat in conventional soybean meal. ESBM has greater energy content than the conventional soybean meals, and has the potential to be an excellent feed ingredient.

Therefore, the aim of the present study was to monitor the effect of supplementing two energy sources (MEGALAC or full-fat soy) on milk production, milk composition, fatty acid profile, and reproductive performance of dairy cows during their early lactation.

Materials and methods

Location and materials

The present study was carried out at the Milk Production Project, Department of Animal and Fish Production, Faculty of Agriculture, Alexandria University. All analyses were carried out at the Animal Nutrition Laboratory in the same department.

MEGALAC® (VOLAC ingredients SDN, BHD, Malaysia) is a palm fatty acid distillate produced as a calcium salt, which allows high levels of oil to be incorporated into ruminant feeding. MEGALAC contains 84% oil, 9% calcium, 5% moisture and 32 MJ/ Kg DM. It contains a fatty acid profile of 44% C16 palmitic acid, 40% C18:1 oleic acid and 9.5% C18:2 linoleic acid. MEGALAC is prepared from crude palm oil that contains high levels of Polyunsaturated Fatty Acid (PUFA).

Full-fat soy is an extruded/expelled processed SBM, provided by Tutweer Animal Nutrition Products Co., Shareholders' Company Giza, Egypt.

Animals and management

Eighteen Holstein dairy cows weighing 412.9 ± 2.8 kg, with mean parity of 2.11 ± 1.01, were randomly assigned into two equal homogenous groups (9 cows per group). Each group was located in an open semi-shaded barn. All cows were diseases-free with a healthy appearance. The control cows, which supplemented with 600 g of megalac/cow were these cows raised under conventional management in the farm. The concentrate mixture and green clover or corn silage were offered three times daily at 6:00, 12:00 and 18:00. The concentrate mixture and green clover or corn silage refusal were recorded daily to record the feed intake. The animals had free access to fresh water and salt blocks (*ad libitum*). Cows were milked three times daily at 5:00, 11:00 and 19:00.

Experimental design

The experiment lasted for twelve weeks. Post parturition, cows were fed 15 kg/cow/day of one of two experimental concentrate mixtures, differing in their fatty acid contents. Additionally, 50 kg/cow/day green clover (*Trifolium alexandrinum*)

or 20 kg/cow/day corn silage were offered to the cows according to the NRC (2001) [18]. The green clover was replaced by corn silage during the summer season in May till August.

Two concentrate mixtures were formulated to be isocaloric and isonitrogenous. The first concentrate mixture of the control group contained 10% soybean meal plus 600 g MEGALAC/cow/d, while the second mixture of full-fat soy group was formulated by replacing 10% of soybean meal and MEGALAC with full- fat soy. Ingredients of the concentrate mixtures and the chemical analyses on dry matter basis of the experimental concentrate mixtures diets, green clover, corn silage, and full fat-soy are presented in Table 1. The fatty acid profile of full-fat soybean and MEGALAC is presented in Table 2.

Table 1: Ingredients of the concentrate mixture and chemical composition on dry matter basis of the two concentrate mixtures, full fat soy, corn silage and green clover fed to dairy cows.

				Concentrate mixtures, %	
Ingredient				**MEGALAC group**	**Full- Fat Soy group**
Cracked Yellow corn				25.0	25.0
Wheat bran				30.0	30.0
Cotton seed meal				20.0	20.0
Sunflower meal				11.5	11.5
Soybean meal				10.0	-
Full-fat soy				-	10.0
Limestone				02.0	02.0
Sodium chloride				01.0	01.0
Trace minerals				0.50	0.50
Item					
Chemical composition	**Green clover**	**Corn silage**	**Full-fat soy**	**MEGALAC group**	**Full- Fat Soy group**
Organic matter, %	88.7	90.6	93.9	93.7	93.5
Crude protein, %	14.8	08.6	43.2	18.7	18.1
NDF, %	52.8	48.0	10.5	41.0	41.9
ADF, %	45.9	39.6	07.6	26.4	27.1
Hemicellulose, %	06.9	08.4	02.9	14.6	14.9
NFC, %	19.4	31.9	29.3	29.9	28.7
EE, %	1.74	2.15	10.96	4.21	4.84
TDN, %	55.7	60.1	93.9	69.4	68.9
DE Mcal/ Kg DM	2.42	2.64	4.53	3.03	2.99
ME, Mcal/ Kg DM	1.98	2.16	3.52	2.48	2.45

OM: Organic Matter; CP: Crude Protein; ME: Metabolizable Energy: 0.82×DE; NDF: Neutral Detergent Fibres; ADF: Acid Detergent Fibbers; NFC: Non-Fibber Carbohydrate: 100- (CP+NDF+EE+Ash); EE: Ether Extract; DE: Digestible Energy; TDN: Total Digestible Nutrients; calculated according to NRC, (2001) [18].

Table 2: The fatty acids profile of MEGALAC and full fat soy.

Fatty acid, %	MEGALAC	Full-fat soy
C6:0	0.17	-
C8:0	1.01	0.12
C10:0	0.03	0.07
C11:0	0.04	-
C12:0	0.32	-
C13:0	0.24	0.05
C14:1	0.12	0.22
C14:0	1.04	0.10
C15:1	0.04	0.41
C15:0	0.11	0.11
C16:1	0.10	-
C16:0	37.60	0.17
C17:0	0.07	11.03
C18:0	8.33	-
C18:1	11.38	-
C18:2	29.41	-
C20:5	0.06	41.19
C20:2	0.06	-
C20:1	0.16	-
C20:0	0.25	-
C22:6	0.05	-
C21:0	-	0.55
C22:0	-	0.24
C20:3∞3	-	22.93
C20:4	-	22.75
Total saturated FA	49.21	12.44
Total unsaturated FA	41.40	87.50

Chemical analysis of feeds

Concentrate mixture, green clover, and corn silage samples were collected biweekly and dried to determine DM content for estimating DMI in each pen. The dried samples were ground to pass a 1 mm-screen using Wiley mill. The ground samples were analyzed for DM by drying a 2.0 g sample in duplicates at 105 °C in a conventional oven for 24 h. For ash determination, a duplicate sample (2.0 g) was incinerated at 600 °C for 2 h in a muffle furnace (Method 942.05) [19], EE was determined according to the AOAC method (Method 920.39) [19], and NDF and ADF were determined according to Van Soest et al. (1991) [20].

Milk yield and composition

Milk yield for individuals was determined once per week. Milk samples were collected weekly and analysed immediately for fat, protein, lactose, Solids-Not Fat (SNF), and ash using the infrared method by Milk Analyzer (MilkoTtester Instruments Inc., Bulgaria). Average fat and protein yields were calculated by multiplying milk yield by fat and protein percentages, respectively on an individual cow basis. Energy Corrected Milk (ECM, MJ/Kg) was calculated on an individual cow basis using the following equation:

ECM = [(0.327×milk yield) + (12.95× fat yield) + (7.2× protein yield)] according to Tyrrell and Reid (1965). Four percentage fats corrected-milk (FCM, kg/day) was calculated using the following equation: FCM = [(0.4×MY) + (15×FY)] according to Gaines and Davidson (1923) [21]; where MY=Milk Yield, FY= Fat Yield.

Synchronization of ovulation and timed insemination

Within the first 60 days postpartum, all cows were synchronized for ovulation using the Ovsynch protocol that consisted of two injections of 2.5 ml GnRH analog equivalent to 10 µg Buserelin (Receptal®, Intervet International B.V. Boxmeer, Holland) 9 days apart and one injection of 2 ml PGF2α equivalent to 500 µg Cloprostenol (Estrumate®, Schering-Plaugh Animal Health, Germany) was given 48 h prior to the second injection of GnRH. Cows were inseminated artificiality with frozen-thawed semen of fertile bull, 16-20 h after the second injection of GnRH (TAI= Timed Artificial Insemination). Cows that failed to be pregnant following the first synchronization were subjected to re-synchronization using the same protocol.

Ultrasound examination

Ovarian activities and pregnancy diagnoses were examined by using ultrasonography examination. A real time B-mode scanner (FALCO) equipped with multi frequency (5 and 7.5 MHz) linear array probe was used (Falco 100, Esaote, The Netherland). Cows were deprived from feed for 12 h before examination. The probe was pushed gently through the rectum until the anechoic content of the bladder become visible on the monitor then the probe was rotated 90˚ clockwise and 180˚ counter clockwise across the reproductive tract until both ovaries and uterine horns were scanned. To determine ovarian structures, ovaries of each cow were scanned at the time of each injection of the synchronization protocol. Size and location of the follicles and corpora lutea on the ovaries were mapped and recorded. The total number and diameters of follicles within each ovary were recorded. Follicles were classified into three categories according to their sizes; small (< 5 mm), medium (5-9 mm) and large (> 9 mm). Pregnancy diagnosis was carried out at day 35 post insemination. Detection of an anechoic uterus, allantois fluid and embryo were considered positive signs of pregnancy.

Blood samples collection

Blood samples were collected via the coccygeal venepuncture using non-heparinized tubes from all cows prior to morning feeding at days 10, 30, 50 and 60 postpartum for biochemical parameters assays. Also, blood samples were collected at the time of each injection of the synchronization protocol (day 60: 1st GnRH injection; day 67: PGF2α injection; and day 69: 2nd GnRH injection) for progesterone assay. Serum samples were obtained by blood centrifugation at 3000 rpm for 20 min., serum samples were stored at -20°C until assayed.

Chemical analysis of serum

Biochemical constituents and hormones were determined to evaluate the physiological status and reproductive performance of animals used in this study. Serum biochemical constituents; i.e. total protein, albumin, urea, creatinine, glucose, triglycerides, cholesterol, Beta-Hydroxybutyrate (BHB) and Non-Esterified Fatty Acids (NEFA) were determined calorimetrically using ultraviolet/visible light spectrophotometer. These parameters were determined using commercial colorimetric kits (Stanbio Diagnostic Company, Germany) for the above mentioned parameters.

Globulin content of each serum sample was obtained by subtracting albumin contents from the corresponding serum total protein concentration. Enzyme-Linked Immune Absorbent Assay (ELISA) was used to measure serum progesterone concentration by commercial kits (DiaMetra, Italy). The lower detection limit was 0.1 ng/mL. The range of the standards used was 0.0-40.0 ng/mL. The intra- and inter-assay coefficients of variations were 4.0 and 9.3%; respectively.

Rectal temperature and pulse rates determination

Rectal temperature and pulse rates of cows were biweekly recorded in the morning (08:00 am). Rectal temperature (RT, °C) was measured using clinical digital thermometer that was gently introduced and attached to rectal wall until a fixed reading was obtained. Pulse rate (PR: pulse/minute) was measured manually via jugular vein.

Fatty acid profile of milk

Five mL of a homogenized milk sample was mixed with 25 ml of methanol and chloroform mixture (2:1) in the separation funnel. The mixture was shaken in a circular ways five times at least, then inverted up and down several times for 5 min and left for separation. For removing turbidity in the milk samples, sodium chloride (0.2 %) was added to the sample in the separation funnel in order to dissolve the turbidity and get the extraction clear. After allowing the layers to separate, the lower layer will be passed in a syringe containing cotton layer at the bottom and 2-3 cm of sodium sulphate and the extract containing chloroform and fat was received in screwed tubes. Chloroform was evaporated through passing nitrogen gas from a nitrogen cylinder into the screwed cap tubes. Ten ml of 1% sulphuric acid with methanol mixture (1:100) was added to the fat into the screwed tube and thereafter two ml of benzene was added to the tube. The tube was kept in the oven at 90 °C for 90 min. One ml of the distilled water and 1ml of benzene were added to the tube and shaken well. A syringe withdrew the clear methanol layer. The fat was passed in a syringe containing cotton layer at the bottom and sodium sulphate. The received fat was injected in a Gas-Chromatograph (GC) for fatty acids profile analysis [22]. Fatty acid methyl esters were quantified using Gas Chromato-

graph Detector (GCD) system (HP6890; Hewlett Packard, Avondale, PA) equipped with a SP-2560 fused silica capillary oven temperature was initially set at 80°C, then ramped at 2 °C/min to 190°C and maintained for 15 min. Inlet and detector temperatures were 250°C and the split ratio was 100 :1. The hydrogen carrier gas flow rate was 1 ml/min, hydrogen flow to the detector was 25 ml/min, air flow was 400 ml/min, and the flow of nitrogen make-up gas was 45 ml/min. Fatty acid peaks were identified using pure methyl ester standards of different fatty acids (NU-Chek Prep, Elysian, MN).

Statistical analysis

Results of the present study were analysed as repeated measures using the mixed procedure of SAS (2002) [23]. The MIXED procedure of SAS (Version 9.2) and the model included effect of treatment, time of sampling and their interactions were tested in a repeated measure design using the following model: $Y_{ijk} = \mu + ES_i + T_j + (ES)_{ij} + Akt + e_{ijk}$; Where: μ is overall mean, ES_i is a fixed effect of the treatment, T_j is a fixed effect of the time, ES_{ij} is an interaction between treatment and time, Akt is random effect of the animal (within treatment) and e_{ijk} is random error assumed to be independent and normally distributed with a mean = 0 and variance = σ^2. Multiple comparisons among means were carried out by the Duncan Multiple Range Test (DMRT; [24]), considering $P < 0.05$ as the significant level.

Results

Effect of dietary fat source on dry matter intake, milk yield and composition and fatty acids profile

Effects of different dietary fat sources in the dairy cow's diet on dry matter intake, milk production, and composition are shown in Tables 3, 4 and 5, respectively. The mean value of dry matter intake slightly increased (P > 0.05) in the MEGALAC group compared with Full- Fat Soy group cows. Moreover, feed efficiency was enhanced (P=0.07) in the MEGALAC group compared with Full- Fat Soy group. Time of the treatment had significant (P < 0.05) effects on feed efficiency but no significant interaction was found. Diet containing MEGALAC did not enhance (P > 0.1) milk yield, fat corrected-milk and energy corrected-milk compared with the diet containing full-fat soy. Contrariwise, time exhibited significant effects (P < 0.05) on milk yield, 4% FCM, ECM, but these parameters were not affected by the treatment × time interaction.

There were no obvious effects due to treatments on milk composition (i.e. fat, protein, lactose, Total Solids (TS), Solids-Not Fat (SNF) and ash). Even though, percentages of SNF, and lactose revealed slight enhancements in the Full- Fat Soy group compared to MEGALAC group. On the contrary, time had significant effects (P < 0.001) on the percentages of SNF and lactose; and on the yields of SNF, lactose, protein and total solids. The treatment and time interaction had significant effects (P < 0.05) on the percentages of protein and lactose (Table 4). However, yields of the SNF, lactose and total solids were not affected (P > 0.05) by treatment. Evidently, the fat/ protein ratio was the sole parameter that has been increased (P < 0.05) in the Full- Fat Soy group compared to the MEGALAC group. Meanwhile, time expressed a significant increase (P < 0.05) of the fat/ protein ratio in the Full- Fat Soy group compared to the MEGALAC group (Table 4).

Fatty acids profiles of early lactating dairy cows fed on the two diets are presented in Table 5. Neither diet has altered the fatty acid profiles in milk. However, Full- Fat Soy diet tended to reduce (P < 0.1) capric acid (C10:0) and to elevate (P < 0.1) oleic acid (C18:1). Concentrations of omega 3 fatty acids were higher (P < 0.05) in the MEGALAC group compared with Full- Fat Soy group; while the ratio of omeg-6/ omega-3 was lower (P < 0.05) in the MEGALAC group than Full- Fat Soy group.

Table 3: Effect of different dietary fat sources (MEGALAC or full-fat soy) on dry matter intake, milk yield, composition and feed efficiency in dairy cows (LSM ± SEM).

Variable	Megalac	Full-fat soy	SEM	P - value		
				Treat.(T)	Time (Ti)	T×Ti
Dry matter intake, kg/d	20.19	19.59	1.67	0.198	-	- -
Milk Yield, kg/d	22.99	19.48	1.681	0.142	<0.001	0.144
ECM[1],kg/d	25.12	21.34	1.8	0.141	<0.001	0.912
4% FCM[2], kg/d	23.71	20.11	1.724	0.142	<0.001	0.906
Feed efficiency, kg milk/kg DMI	1.12	1.04	0.01	0.075	-	-

[1]ECM: Energy Corrected Milk was calculated using [(0.327* MY)+(12.95*FY)+(7.2*PY)] according to Tyrrell and Reid, (1965) [25]; [2]FCM: Fat Corrected Milk was calculated using [(0.4* MY)+(15*FY)] according to Gaines and Davidson,(1923) [21].

Table 4: Effect of different dietary fat sources (MEGALAC or full-fat soy) on milk composition of dairy cows (LSM ± SEM).

Variables	Megalac	Full-fat soy	SEM	P - values		
				Treat. (T)	Time (Ti) (Ti)	T × Ti
Fat, %	4.08	4.14	0.102	0.65	0.88	0.574
Fat yield, kg/d	0.96	0.84	0.08	0.285	0.25	0.574
Protein, %	3.03	3.01	0.038	0.531	0.239	0.054
Protein yield, Kg/d	0.7	0.6	0.059	0.247	0.044	0.846
Lactose, %	4.41	4.5	0.041	0.111	<0.001	0.033
Lactose yield, Kg/d	1.01	0.88	0.071	0.178	<0.001	0.115
SNF, %	8.2	8.33	0.06	0.127	0.005	0.304
SNF yield, kg/d	1.89	1.62	0.137	0.171	<0.001	0.199
Total solid, %	12.3	12.42	0.122	0.475	0.402	0.137
Total solid yield	2.92	2.48	0.213	0.12	<0.001	0.631
Ash, %	0.2	0.8	0.032	0.787	0.987	0.558
Fat/Protein ratio	0.60b	0.63a	0.007	0.018	0.258	0.831

[a],[b] Means with different superscript letters are different at $P < 0.05$.

Table 5: Effect of different dietary fat sources (MEGALAC or full-fat soy) on fatty acid composition (%) of milk (LSM ± SEM).

Fatty acid, %		Megalac	Full-fat soy	SEM	P - value
Caproic acid	C6:0	0.025	0.018	0.010	0.771
Caprylic acid	C8:0	0.177	0.119	0.023	0.216
Capric acid	C10:0	0.204	0.105	0.027	0.067
Undecylic acid	C11:0	0.236	0.194	0.043	0.646
Lauric	C12:0	0.825	0.693	0.086	0.470
Tridecylic acid	C13:0	1.076	0.986	0.200	0.832
Myristoleic acid	C14:1	0.978	0.626	0.127	0.177
Myristic	C14:0	8.496	8.066	0.303	0.500
Pentadecyloleic acid	C15:1	1.875	1.513	0.219	0.431
Pentadecylic acid	C15:0	4.573	3.124	0.617	0.257
Palmitoleic acid	C16:1ω-7	1.772	1.281	0.487	0.634
Palmitic	C16:0	29.22	28.42	1.118	0.736
Margaroleic acid	C17:1	1.713	2.355	0.225	0.167
Margaric acid	C17:0	0.718	0.913	0.070	0.180
Medium chain fatty acid[(1)]					
α-Linolenic acid	C18:3 ω-3	3.260	0.635	0.829	0.120
Linoleic acid	C18:2C ω-6	12.55	15.06	1.236	0.330
Oleic	C18:1C ω-9	2.143	7.033	1.424	0.088
Stearic	C18:0	19.75	19.79	1.351	0.989
Eicosapentaenoic acid	C20:5 ω-3	0.000	0.027	0.012	0.271
Eicosatrienoic acid	C20:3 ω-3	0.366	0.203	0.124	0.524
Gondoic acid	C20:1	0.002	0.583	0.187	0.127
Arachidic acid	C20:0	1.507	1.307	0.230	0.682
Heneicosylic acid	C21:0	0.874	2.074	0.725	0.431
Erucic acid	C22:1 ω-9	7.801	4.750	2.002	0.469
Long chain fatty acid [(2)]					
Total saturated FA		67.68	65.80	3.244	0.662
Total unsaturated FA		32.32	34.20	3.244	0.662
Mono unsaturated FA		16.28	18.14	5.263	0.756

Poly unsaturated FA	15.94	16.06	2.291	0.934
ω-3	3.621	0.860	1.128	0.089
ω-6	12.55	15.06	2.157	0.330
ω-6: ω-3 ratio	3.46[b]	17.51[a]	1.549	0.030
ω-7	1.772	1.281	0.487	0.634
ω-9	9.94	11.78	1.426	0.557

[a],[b] Means with different superscript letters are different at P < 0.05,
(1) medium chain: from C_{14} to C_{17}; (2) long chain: ≥C_{18}.

Effect of dietary fat source on serum metabolites

The effect of inclusion of Full-Fat Soy or MEGALAC in the diet to lactating dairy cow's diets at their early lactation on nitrogenous serum metabolites is presented in Table 6. The results revealed non-differences between the two tested diets on serum total protein concentration. Serum protein concentration increased numerically at d 10 up to d 30 postpartum then decreased at d 60 postpartum in both groups. Full-Fat Soy diet significantly (P < 0.05) increased serum albumin than this found in MEGALAC diet. Serum albumin numerically increased from d 10 up to d 30 postpartum then decreased at d 50 postpartum in both groups. Serum globulins concentration was found to be lower in Full-Fat Soy group compared to the MEGALAC group. Time had a significant (P < 0.05) impact on serum globulins concentration, whereas a non-significant effect was found due to the treatment × time interaction. Full-Fat Soy diet significantly (P < 0.05) increased albumin/ globulin (A/G) ratio, while no time × treatment interaction was detected on A/G ratio. The time had shown a significant (P < 0.05) effect on A/G ratio.

Serum concentration of urea has been reduced (P < 0.05) in Full-Fat Soy group compared with MEGALAC group. Time-wise, urea concentrations have shown numerical increases at d 10 up to d 30 which thereafter decreased at d 60 postpartum in both groups. Inversely, a non-significant difference (P > 0.05) was found between the two diets on serum creatinine. As for the time of blood sampling in the MEGALAC group, there exist numerical increases in serum creatinine starting at d 10 up to d 30, decreased at d 50, and increased again at d 60 postpartum. On the other hand, an opposite trend was found in Full-Fat Soy group showing a decrease in serum creatinine at d 10 up to d 30 and increased thereafter at d 50 postpartum.

Effect of inclusion the full-fat soy or MEGALAC to lactating dairy cow's diet at an early lactation on the energetic serum metabolites are presented in Table 7. Energy-yielding metabolites including glucose, triglycerides and cholesterol were modulated by the treatments. The concentration of serum glucose was not affected by treatment, time or interaction. Serum triglycerides approached significantly higher (P = 0.059) levels in MEGALAC group than in Full-Fat Soy group. Time of blood sampling also exhibited a significant (P < 0.05) effect on triglycerides. In MEGALAC group there found numerical elevations starting at d 10 up to d 60 postpartum, while in Full-Fat Soy group there found numerical increases at d 10 up to d 30 and a slight decrease at d 50 which increased thereafter. Serum cholesterol increased (P < 0.05) in Full-Fat Soy group compared with MEGALAC group. Neither the time of blood sampling nor the interaction has shown significant changes in serum cholesterol. Serum concentrations of both NEFA and BHB were not altered in both groups. Also, neither the time of blood sampling nor the interaction has shown significant changes in serum NEFA. Time and treatment × time interaction have shown significant (P < 0.05) effects on BHB in serum. The temporal changes of BHB in both groups revealed increases at d 10 up to d 30 followed by a decrease at d 50 postpartum.

Table 6: Effect of different dietary fat sources (MEGALAC or full-fat soy) on nitrogenous blood biochemical attributes in dairy cows (LSM ± SEM).

Variables	Megalac	Full-fat soy	SEM	P - Values		
				Treat (T)	Time(Ti)	T × Ti
Total protein, g/dL	9.1	8.48	0.28	0.555	0.093	0.796
Albumin, g/dL	4.06[b]	5.05[a]	0.213	0.001	0.196	0.538
Globulin, g/dL	5.04[a]	3.43[b]	0.323	0.012	0.015	0.434
A/G ratio	0.81[b]	1.47[a]	0.206	<0.001	0.022	0.027
Urea, mg/dL	38.07[a]	30.23[b]	2.585	0.036	0.253	0.195
Creatinine mg/dL	2.12	1.94	0.18	0.481	0.027	0.371

[a],[b] Means with different superscript letters are different at *P*< 0.05.

Table 7: Effect of different dietary fat sources (MEGALAC or full-fat soy) on energetic blood biochemical attributes in dairy cows (LSM ± SEM).

Variables	Megalac	Full-fat soy	SEM	P - Values		
				Treat (T)	Time (Ti)	T ×Ti
Glucose, mg/dL	33.71	33.52	1.267	0.915	0.366	0.463
Triglycerides, mg/dL	16.76[a]	13.04[b]	1.334	0.059	0.047	0.74
Cholesterol, mg/dL	211.28[b]	255.64[a]	14.21	0.028	0.708	0.835
NEFA, mmol/L	0.69	0.66	0.073	0.76	0.71	0.962
BHB, mmol/L	1.03	0.96	0.085	0.569	0.053	<0.001

[a],[b] Means with different superscript letters are different at *P*< 0.05.

Effect of dietary fat sources on rectal temperature and pulse rate

Rectal temperature and pulse rate values are presented in Table 8. There were no changes in pulse rate due to treatment or treatment × time interaction, rather a significant (P < 0.05) effect was attributed to the time of blood sampling. On the other hand, a slight increase (P < 0.10) in Rectal Temperature (RT) was recorded in Full-Fat Soy group (38.72 °C) compared with 38.6°C in MEGALAC group. Time of blood sampling has shown significant (P < 0.01) changes in rectal temperature. Meanwhile, there was a non-significant effect due to the interaction on RT.

Table 8: Effect of different dietary fat sources (MEGALAC or full-fat soy) on rectal temperature and Pulsation rate in dairy cows (LSM ± SEM).

Variable	Megalac	Full-fat soy	SEM	P - Values	Time (Ti)	T×Ti
				Treat. (T)		
Pulsation rate (pulse/minute)	69.24	68.52	0.567	0.375	0.035	0.495
Rectal temperature, °C	38.6	38.72	0.048	0.075	0.003	0.334

Effect of dietary fat source on ovarian activity and reproductive traits

Data of the effect of dietary fat source on the ovarian structures, serum progesterone concentration, and conception rate in estrous-synchronized dairy cows are shown in Table 9. MEGALAC-containing diet increased (P < 0.05) the number of small ovarian follicles resulting in 1.15 in MEGALAC group compared with 0.33 follicles in Full- Fat Soy group. On the other hand, the mean number of medium follicles showed similar values in both treatments. However, the number of large follicles exhibited an opposite trend to what was found for small follicles, showing an increase (P = 0.06) in the Full- Fat Soy group (1.12) compared with MEGALAC group (0.92).

Table 9: Effect of dietary fat source (MEGALAC or full-fat soy) on ovarian structures, peripheral progesterone concentration and conception rate in estrous-synchronized postpartum dairy cows (LSM ± SEM).

Variables	Megalac	Full-fat soy	SEM	P- Values		
				Treat. (T)	Time (Ti)	T×Ti
Total number of follicles	2.4	1.83	0.292	0.148	0.89	0.72
Follicles category						
Small <5mm	1.15[a*]	0.33[b]	0.257	0.038	0.404	0.296
Medium >5-<9mm	0.34	0.37	0.154	0.786	0.052	0.899
Large ≥9mm	0.92	1.12	0.122	0.069	0.775	0.208
Diameter of largest follicle, mm	12.11	13.4	0.737	0.235	0.384	0.854
Count of corpora lutea,	0.29	0.2	0.076	0.144	<0.001	0.706
Diameter of corpora lutea, mm	17.65	16.44	1.36	0.685	0.652	0.972
Progesterone concentration, ng/ml	1.43	1.3	0.334	0.803	0.638	0.775
Conception rate, %						
First service	33.33 (3/9)	44.44 (4/9)	-	0.629	-	-
Second service	33.33 (2/6)	60.00 (3/5)	-	0.377	-	-
Total conception rate	55.55 (5/9)	77.78 (7/9)	-	0.317	-	-

*a,b Means with different superscript letters are different at P < 0.05.

Neither numbers of CL nor its diameters have shown a difference among treatments. Moreover, serum progesterone concentration was similar in both groups. Ultimately, even though there were non-significant differences in the conception rate among treatments, there still a better conception rate achieved by the Full- Fat Soy group.

Discussion

Dry matter intake and feed efficiency

The NEB occurs in dairy cattle during peak lactation, and they cannot consume enough energy to compensate for increased milk production and subsequent energy loss. If the energy density of the diets is not increased, then the lactating cattle will begin to lose weight and will not be capable of producing high quantities of milk [7]. One major concern of the present study was to minimize the occurrence of NEB of dairy cows during the early lactation period. It has been reported that fat supplement improved the energetic efficiency because it reduced loss of energy in the form of heat, methane, and urine [26]. Similarly, cows fed on the heat-treated full fat soy recovered their postpartum weights earlier than cows fed on soybean meal, and row soy [27].

In this study, cows in both groups consumed the same amount of feed as denoted by the similar value of the dry matter intake. However, it could be observed that the feed efficiency was better (P = 0.07) in the MEGALAC-fed cows. This could be due to the increase in fat digestibility when protected fat is included in the diet [28]. These authors suggested that fat digestibility value was high because the added fat is characterized by greater digestibility and availability than fatty acids within feed particles, and because endogenous fat losses are diluted by the increment in the level of fat in the ration.

The mode of action affecting DMI depression from feeding Ca-SFA to lactating cows has been identified in three possible areas: Palatability and ruminal and gastrointestinal motility effects. Grummer et al. (1990) [29] determined the palatability effects of four different fat products (sodium alginate encapsulated dry tallow, tallow, FFA, and Ca-SFA) on four dairies involving 209 lactating dairy cows. The authors observed that cows displayed a better intake of fat products, except Ca-SFA similar to what is recorded in the current study. This observation was important because it indicated a possible inhibitory mechanism beyond palatability or general adaptation that led to a continued and prolonged DMI depression.

A second possible mode of action regarding DMI depression from the inclusion of Ca-SFA is the disruption of ruminal fermentation because of the effects of unsaturated fatty acid. Although Ca-SFA were observed to be inert in the rumen *in vitro*, Wu et al. (1991) [30] reported that 58% of unsaturated 18-carbon fatty acids in Ca-SFA were biohydrogenated *in vivo*. The researchers stated that biohydrogenation could only occur after dissociation of the Calcium salt. Hawke and Silcock (1969) [31] had similar findings and concluded that a free carboxyl group of the fatty acid was required for bio hydrogenation to proceed. Consequently, the actual case is that Ca-SFA not inert in the rumen. Therefore, the negative effects of unsaturated fatty acids on rumen fermentation are probable.

The third possible mode of action regarding DMI depression in cows fed on Ca-SFA is the effect on gastrointestinal motility. Bauman and Griinari (2003) [32] noted that digestibility does not differ significantly between C16 and C18 saturated FA, and is less for longer chain saturated fatty acids as compared with PUFA. Later researchers further noted that differences in digestibility among individual fatty acids contribute very little to the extensive variation (~60 to 90%) in the digestibility of dietary lipids, and that the majority of this variation reflects differences among individual experiments, differences in diets, and to specific feed components.

Metabolism and response to heat stress

Stress in any form, external or internal, greatly influences the productivity and well-being of dairy animals. The genetic selection of stress-resistant animals, optimum nutrition, and regular monitoring of animals' health during periods of critical stress may help to reduce stress in dairy animals [33]. In addition, heat stress hampers nutrient utilization and production of animals, and dietary betaine supplementation at 50 g/ d/animal can mitigate the adverse effects of heat stress, protects intestinal integrity, enhances nutrient utilization and improves growth performance of growing heifers during heat stress [34].

In the present study, changes in lipid profile were detected; it is interesting to note that the concentrations of serum cholesterol and triglycerides were different between the MEGALAC group and Full- Fat Soy group with an opposite trend.

Bailoni et al. (2004) [35] found no differences in these metabolites when soybean meal or full-fat soy was used. Accordingly, differences obtained in this study in triglyceride concentrations may be due to the effect of inclusion of MEGALAC in the diet. MEGALAC, a protected fat, may increase the availability of blood free fatty acids; those are essential components of triglycerides [36]. On the other hand, the fatty acid analysis of full-fat soy showed its low content from saturated fatty acids; i.e. C12:0 (lauric acid), C14:0 (myristic acid) and C16:0 (palmitic acid), especially those known to increase plasma Low Density Lipoprotein (LDL) and cholesterol concentrations [37]. This contradiction may be due to the occurrence of rumen bio-hydrogenation to unsaturated fatty acids in the full-fat soy, preventing its biological expected effects.

Bailoni et al. (2004) [35] found that the replacement of about 70% of soybean meal with extruded or toasted full-fat soybean seeds in diets for lactating cows did not affect metabolic profile including total protein, urea, glucose, cholesterol, NEFA, and triglycerides. Similarly, in the present study complete replacement of soybean meal with extruded full-fat soy did not affect concentrations of serum glucose, NEFA, BHB, creatinine and total protein. However, the concentration of serum albumin increased in the full fat soy-fed cows. The reason for this increase needs to be further investigated.

Metabolic status and disease condition of cows during the transition period could be assessed by haptoglobin (Hp), Serum Amyloid A (SAA) and energy-related parameters [38]. Inflammatory cytokines (TNF-α, IL-6, IL-10), as well as acute phase proteins (SAA, Hp), have been reported as novel diagnostic biomarkers for many diseases that occur around the transition period and during early lactation [39]. Plasma Non-Esterified Fatty Acids (NEFA) are associated with various periparturient diseases because an inflammatory condition leads to metabolic disturbance and subsequently negative energy balance around parturition [40].

Higher levels of inflammatory biomarkers e.g. cytokines IL-6 and TNF-α, plasma amyloid A (SAA), Acute Phase Proteins (APPs) and NEFA along with lower levels of thyroid hormones

in cows calving during the summer season indicate the intensity of physiological and environmental stress experienced by them. Understanding the inflammatory and metabolic responses around the calving period could help to predict the most susceptible risk time for disease incidence so that efficient management protocols can be made to avoid the health issues occurring during the transition period and extreme weather conditions [41].

The significant increase in the concentration of serum urea observed in the present study adherent to the MEGALAC group is consistent with the findings of Loor et al. (2005) [42] and Otaru et al. (2011) [43] on lactating Holstein cows supplemented with linseed oil, and also in Red Sokoto goats supplemented with palm oil. In the present study, MEGALAC inclusion reduced the amount of non-structural carbohydrate that may decrease the ingestion and availability for degradation in the rumen. According to Lykos et al. (1997) [44], when the rate of degradation of total non-structural carbohydrate was reduced, high producing Holstein cows had high blood urea-nitrogen and milk urea-nitrogen concentrations. Animals which had the least amount of ingested non-structural carbohydrate significantly had higher plasma urea nitrogen.

In this study, an additional benefit of the inclusion of protected fat in the diet was observed, since the rectal temperature of MEGALAC group cows was lower than that determined in the full-fat soy fed-cows. Previous works showed conflicting results, whereas, rumen-protected fats allow the inclusion of a substantial quantity of fat in the diet, which could lower heat increment significantly. Also, Holter and Young (1992) [45] found that the addition of 15% whole cottonseed, or 15% whole cottonseed plus 0.54 kg of calcium salts of fatty acids resulted in heat production in excess of maintenance declined by 6.7 and 9.7%, and total heat loss declined by 4.9 and 7.0%, respectively. The high-fat levels produced a measurable decline in heat production in the thermoneutral conditions. On the contrary, Knapp and Grummer, (1991) [46] offered diets supplemented with 5% fats (60% prilled fatty acids and 40% tallow) to cows held at thermoneutral or heat-stressed environment and reported a non-significant increase in milk yield of 1.1 and 1.3 kg in the cool and hot environments, although fat-corrected milk increased by 2.7 and 1.8 kg/d, respectively. No diet by environment interaction was reported.

Vaibhav et al. (2018) [47] found that the combined supplementation of protected fat, yeast, niacin, zinc and chromium in the ration of growing heifers can mitigate the detrimental effects of summer stress under tropical conditions which are indicated by the higher average daily gain, body measurements and IGF-1 values in the treatment group.

Milk yield, composition, and fatty acid profile

In the present study, no differences in milk yield, 4%-fat corrected milk, and energy corrected-milk were observed due to replacement of either soybean meal plus MEGALAC or extruded full-fat soy. Similarly, Bailoni et al. (2004) [35] reported that feeding dairy cows with full-fat extruded or roasted soybean seeds as replacement of soybean meal had no effects on milk yield or its composition. Also, Ure et al. (2005) [48] found that feeding cows twice with extruded soybean meal or twice extruded soybean meal treated with calcium oxide alone or with calcium oxide plus lingo sulfonate did not improve feed intake, milk yield, or milk composition. One supposed beneficial effect of feeding extruded full-fat soy is to increase the Rumen Undegraded Protein (RUP). Increasing RUP content of protein feeds with inherently high degradation rates can have a positive influence on milk production and milk composition in high-producing dairy cows by allowing a greater flow of essential amino acids available for absorption in the small intestine [18].

In the current study, milk composition did not differ between the two experimental groups. This result is relatively in accordance with that obtained by Radivojević et al. (2011) [49] in which partial replacement, but not a complete replacement, as in the present study; of extruded full-fat soy with soybean meal did not change the percentage of milk fat or protein. Similarly, Scott et al. (1991) [15] found no effects on milk composition when different soybean products (full-fat soy or extruded) were used.

In the present study, feeding on extruded Full-Fat Soy group significantly decreased protein/ fat ratio (i.e., increased fat percentage) in milk compared with MEGALAC group. On the contrary, Eifert et al. (2006) [50] detected a decrease in milk fat content, from 3.34 to 3.13% when soybean oil was included in the mixture of concentrated feed. Also, Radivojević et al. (2011) [49] showed an opposite trend to what was observed in the current study in which the inclusion of extruded full-fat soy increased milk fat/ protein ratio compared to the diet contained both soybean and extruded full fat soy. This increase in the ratio of fat/protein in extruded full-fat soy observed in this study could be related to the significant increase in serum cholesterol. It is interesting to know that the ratio of fat/protein is a valuable indicator of lipo-mobilization and the NEB status in postpartum cows. A fat/ protein ratio in early lactation greater than 2.0 showed an increase in postpartum diseases such as retained placenta, left-displaced abomasum, metritis, and clinical endometritis; but a decrease in the early lactation milk yield [51]. Accordingly, it could be stated that both diets used in the present study were adequate to meet animal energy requirements, since the fat/ protein ratio was less than 2.

As shown in Table 4 extruded full-fat soy had a higher percentage of unsaturated fatty acid compared with MEGALAC. Although, analysis of fatty acid content of milk fat showed that the percentage of milk unsaturated fatty acid in the full-fat soy group hasn't been increased compared with MEGALAC group. This finding presents evidence on the occurrence of rumen bio-hydrogenation for unsaturated fatty acids in extruded full-fat soy, which again may negate the efficiency of processing oilseeds on ruminal lipid digestion by reducing the amount of polyunsaturated fatty acids subjected to biohydrogenation. It has been reported that under normal conditions, unsaturated FA that escapes biohydrogenation in the rumen are selectively esterified to plasma phospholipid and cholesteryl ester fractions. This process makes those FA unavailable for milk fat synthesis. However, as was hypothesized by Hartfoot (1981) [52], when large amounts of PUFA reach the intestines, the mechanism for segregating these components is not adequate, and the excess is incorporated into triacylglycerol. Triacylglycerols are the primary source of preformed FA for milk fat synthesis in the mammary gland [52]. Similarly, in the present study, full-fat soy increased serum cholesterol concentrations, while milk fat and milk ploy unsaturated fatty acids did not alter, supporting the previous hypothesis.

It is interesting to note that, however, the percentage of unsaturated fatty acids in milk was not different in both experimental groups. The ratio between ω-3 to ω-6 unsaturated fatty acids was different, whereas the percentage of ω3 fatty acids

was higher in the MEGALAC group, and this increase was mainly due to the increase in the percentage of C18:3. Consequently, ω-6 / ω-3 ratio was lower in the MEGALAC group than in the Full-Fat Soy group. It is worthy to know that this change in fatty acid profile is of benefits for human health. The issue is not that ω-6 fatty acids are unhealthy; they are considered one of the "good" fats and an important part of a healthy diet. However, high ω-6 can interfere with the way the body utilizes ω-3 fatty acids and thereby limit their health benefits; such as reducing the risk of cardiovascular disease, diabetes, and obesity [53,54].

Reproductive performance

In the present study, the dietary fat sources had a clear effect on the distribution of ovarian follicles. MEGALAC containing-diet improved significantly the overall number of small follicles and also the number of total follicles. Homa and Brown, (1992) [55] studied the fatty acid composition of follicular fluid from small and large developing follicles. Linoleic acid was the major fatty acid in follicular fluid, constituting about a third of total fatty acids. Oleic acid constituted 19% of the total fatty acids in small follicles and 17% in large follicles. Saturated fatty acids accounted for less than 30% of total fatty acids in follicular fluid. The proportion of linoleic acid was significantly lower in follicular fluid from large follicles (31% of total fatty acids) than from small follicles (35% of total fatty acids) and there was a significant inverse correlation between follicle diameter and proportion of linoleic acid in follicular fluid.

Thus, the improved number of small follicles observed in this study may be due to the high percentage of linoleic acid in MEGALAC. Such effects of fat supplementation on ovarian follicle distribution were previously observed [56]. Replacing corn with Ca-LCFA from palm oil in the ration of dairy cows at calving increased the number of medium size follicles (6-9 mm) and follicles greater than 15 mm within 25 days postpartum. The greatest increase in medium follicle populations occurred in response to plant oil consumption, which is likely a direct result of the effects of high levels of linoleic acid in the Rumen [57]. The number of medium size follicles (5 to 10 mm) was higher in beef cows which consumed feed with a greater content of PUFAs [58] and in dairy cows which consumed a diet enriched with 5% n-3 fatty acids derived from fish oil [59]. Similar results were observed in cows fed with diets enriched with n-3 or n-6 fatty acids [60].

On the other hand, extruded full-fat soy significantly increased the overall number of large follicles (ovulatory follicles). It could be observed that, however, the number of large follicles tended to be higher in extruded full-fat soy; this was not associated with an increase in the number of corpora lutea. Ovarian scanning during the synchronization period revealed that the increase in the number of large follicles did not continue until the time of ovulation induction (second GnRH injection). This may explain why this group did not have a better number of corpora lutea when compared with MEGALAC group.

In the present study, cows in both experimental groups had serum progesterone concentration higher than 1 ng/ml, which indicated that the resumption of the ovarian activity (ovulation activity) happened within 60 days postpartum in both groups. Marín-Aguilar et al. (2007) [61] observed that Holstein cows supplemented with plant oil (60% PUFAs) exhibited a reduction of 7 days for the resumption of ovarian activity relative to the control group. Furthermore, De Veth et al. (2009) [62] observed that the time at first ovulation in dairy cows was reduced by 8 days when they were supplemented with 8 g/d/cow of *trans*-10, *cis*-12 CLA. Conception rate in both experimental groups was similar, whereas 55% to 77% of cows were pregnant within 100 days postpartum. The lack of significance may be due to many reasons. First, the conception rate may be mainly dependent on animal body weight rather than the type of dietary fatty acids. Second, the number of animals used in the present study was not adequate to detect statistical differences; whereas conception rate is categorical data [63].

Conclusion

It is of great benefit for the dairy cows, especially during the period during which they produce their peak of milk, i.e. during the 9th week postpartum, to supplement their diets with a proper source of fat, i.e. MEGALAC. The inclusion of this rumen-protected fat in the diet of dairy cows not only maintained its body condition score, but it also enhanced the metabolism, milk quality and quantity. and their reproductive performance.

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