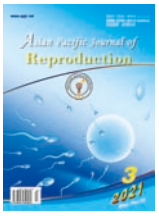

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Effect of flaxseed supplementation on metabolic state, endocrine profiles, body composition and reproductive performance of sows

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ABSTRACT

Objective: To assess the effect of flaxseed supplementation on metabolic profile, endocrine concentrations, non-esterified fatty acids (NEFA), body composition variables, and reproductive performance of sows.

Methods: All the 21 crossbred Large White Yorkshire sows were considered in the study period starting at day 1 of current farrowing when the feeding of specific supplemental ration was started until the day of subsequent farrowing (days 150-155) and were equally allocated into three groups. Group 1 served as the control group and followed their normal feeding schedule. Group 2 and group 3, in addition to their normal feeding schedule, were supplemented with flaxseed at a rate of 0.5% and 1.0% of the dry matter, respectively. Blood samples were collected 15 days prior to farrowing, on the day of farrowing (day 0), at weekly intervals until day 28 of lactation and at monthly intervals during gestation to harvest the plasma. Plasma was used to assess the metabolic and endocrine status of sows. Body weight of each sow and individual birth weight of all piglets born were measured.

Results: Flaxseed supplementation led to decrease in plasma cholesterol and triglyceride levels in the supplemented groups than in the control group (P<0.05). Plasma estradiol-17β level was higher in group 2 than that in group 1 and 3 on day 90 of the gestation period (P<0.05). The mean plasma level of insulin-like growth factor 1 was higher in group 3 than that in group 1 and 2 both in late lactation (day 28) as well as in early gestation (day 30) (P<0.05). Plasma NEFA and weight gain were greater in sows of group 2 and 3 compared to those fed with the normal control diets (P<0.05). The proportion of pregnant sows relative to sows bred was 100.0% in group 2 and 3 and 85.7% in the control group. Piglet mortality was lower in group 2 and 3 compared to group 1 (P>0.05).

Conclusions: Flaxseed improves endocrine profiles, NEFA concentrations and body weight, resulting in better pregnancy rate and litter size.

KEYWORDS: Endocrine profile; Flaxseed; Non-esterified fatty acids; Piglet mortality; Pregnancy rate; Sow

1. Introduction

In India, rearing of pigs for meat purpose is favored due to their better compliance for high prolificacy (10-12 total born piglets per litter) and productivity (nine pigs weaned per litter). The better prolificacy and productivity of this species plays an important role in nutritional security and socio-economic status of rural mass[1]. Primary factor determining productivity of swine industry is the number of pigs weaned per sow per year. This criterion is essentially dependent on the reproductive efficiency of sow[2]. Reproductive efficiency is hampered in sows by prolonged weaning to estrus interval, low farrowing rates, reduced born alive, decreased number of piglets weaned per litter and high number of stillbirths[3], resulting in greater fiscal losses to pig farmers. Nutritional deficit is a key factor contributing to the number of non-productive days in a sow's lifetime and has shown to affect reproduction[4].

Various nutritional strategies through manipulations of dietary feeding regimes have been recommended to improve the reproductive performance of sows[5,6]. Some of these strategies include feeding of polyunsaturated fatty acids (PUFA) during gestation and lactation[7]. In gestation diets, supplementation of PUFA results in accelerated embryo neural development[8] and increased litter size[9]. When supplemented in lactation diets, there is secretion of PUFA in milk to support growth and development of nursing litter[10]. Moreover, diets containing high levels of PUFA affect energy metabolism and lead to reduced plasma

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triglyceride[11] and cholesterol[12] levels. Although much evidence exists that supplemental PUFA during lactation has positive effects on the reproduction of dairy cow[13], data on PUFA requirements for lactating sows are scarce and meager[14]. Few studies[15,16] in sows have shown that prolonged dietary supplementation of two percent PUFA on dry matter basis improved their reproductive performance.

Flaxseed offers high α-linolenic acid content (58% of the total fatty acids), better palatability compared to fish meal as a consequence of non-fishy odor, making it an energy dense replacement compared to other costly feed ingredients[17]. In addition, flaxseed also contains high concentrations of secoisolariciresinol diglycoside, a precursor of lignans, which in turn exhibits estrogenic activities[18]. Hence, the present study was to investigate the impact of minimum dietary concentrations of supplemental flaxseed on the metabolic, endocrine and reproductive performance of post-farrowed sows.

2. Materials and methods

2.1. Animals, experimental design and dietary treatments

Twenty one apparently healthy pleuriparous (2nd to 5th parity) pregnant (>95 days) crossbred Large White Yorkshire maternal-line sows were randomly selected at a pig farm, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana (n=6) and at a private organized pig farm (Polar Pig Breeding Farm, Khasi Kalan, Ludhiana, n=15). Sows at both farms were housed under semi-loose housing system (covered area, 10 feet × 10 feet and uncovered area, 10 feet × 10 feet). All the sows were offered feed twice a day, morning (10.00 a.m.) and afternoon (4.00 p.m.) and had free access to drinking water throughout the day. The present study was conducted during the months of August 2019 through January 2020 with average daily temperature of (23.3±1.1) ℃ and relative humidity of (71.2±1.0)% inside the pig sheds.

All the sows were considered in the study period starting at day 1 of current farrowing when the feeding of specific supplemental ration was started until the day of subsequent farrowing (days 150-155). All the sows were divided equally into three treatments *viz*. group 1, group 2 and group 3 based on parity, body weight at farrowing and number of piglets born. Accordingly, group 1 (n=7) served as the control and followed their normal feeding schedule starting on day 1 of lactation until the day of subsequent farrowing (days 150-155). Sows of group 2 (n=7) and group 3 (n=7), in addition to their normal feeding schedule, had flaxseed supplementation at a rate of 0.5 percent and 1.0 percent of the dry matter[19], respectively, during the study period.

Cross-fostering of piglets was done within a group, if required, immediately after 24 h post-farrowing to allow adequate colostrum intake from sows and to ensure their uniform nourishment. Litter size was standardized to 10 piglets per litter [(10.0±0.2) piglets]. During the experimental period, piglets did not have access to creep feed and/or supplemental milk. In the farrowing pen (one sow/pen/group), all the sows were offered standard lactation diet prepared using maize, soyabean meal, de-oiled rice bran, wheat bran, salt and mineral mixture. Lactation rations were fed individually to sows twice daily at the rate of 4%/kg bodyweight/day/sow, based on body weight starting on day 1 of lactation, throughout the 28-day lactation period (Table 1)[14]. In addition, each sow of group 2 and group 3 was also supplemented with flaxseed during the lactation period (days 0-28). Litters were weaned on day 28 of lactation.

After weaning, sows were moved to a common breeding area and housed as breed groups (2-3 sows/pen/group) in breeding pens. Respective lactation diets same as mentioned above were continued to be fed to all the sows individually, twice a day following weaning at the rate of 3%/kg bodyweight/day/sow (Table 1). In addition, all the sows of group 2 and group 3 were continued flaxseed supplementation. During the first three days after weaning, each sow was provided exposure to a rotation of mature boars once a day for 30 min to facilitate estrus detection. From day 4 until day 7 after weaning, sows were properly checked for heat signs, twice daily. Six to eight hours after the exhibition of physical signs of first standing estrus, the sows were bred with proven fertile boars. If still in estrus, the sows received a second or third mating 24 h after first or second mating. At day 25 after breeding, sows were checked for pregnancy by using ultrasound machine. Sows confirmed pregnant were housed as groups (2-3 sows/pen/group) in gestation pens. During gestation, sows were offered standard gestation diet at the rate of 3%/kg bodyweight/day/sow (Table 1). Amount of feed was rescheduled fortnightly according to the weight of sows. One week prior to expected date of farrowing, the pregnant sows were shifted to individual farrowing pens.

2.2. Blood sampling

Following short-time nose-snare restraint, blood samples (5 mL) were collected into heparinized (1:1 000) polystyrene tubes from each sow through peripheral ear vein. Blood samples were collected fifteen days prior to farrowing (day 15), at farrowing (day 0), at days 7, 14, 21, 28 of lactation period and at days 30, 60 and 90 of gestation period to assess endocrine profile and metabolic status of animals. Blood samples were centrifuged for 15 min at 1 107 ×g to harvest the plasma. The plasma samples were stored at -20 ℃ in duplicate vials until assayed.

2.3. Analysis of plasma metabolites and hormones

Plasma glucose, cholesterol , total protein , triglyceride , calcium , alanine transaminase (ALT), aspartate transaminase (AST), gamma glutamyl transpeptidase (GGT), blood urea nitrogen (BUN) and creatinine were assayed in duplicate by Vitros 350 Chemistry System (Ortho Clinical Diagnostics, Buckinghamshire, England) using kits (Ortho Clinical Diagnostics, NY, USA) validated for multi-species use.

Plasma progesterone, estradiol-17β, insulin like growth factor-1 (IGF-1) and non-esterified fatty acids (NEFA) concentrations were quantified in duplicate using commercial Porcine specific Enzyme

Table 1. Nutritional composition of various diets fed to sows.

Items	Control diet (Group 1)	FS, 0.5% (Group 2)	FS, 1.0% (Group 3)
Ingredient (kg/100 kg)			
Maize	48.0	48.0	47.5
Soybean meal	11.1	11.1	11.1
Groundnut extraction	18.5	18.5	18.5
De-oiled rice bran	10.5	10.5	10.5
Rice polish	10.6	10.1	10.1
Common salt	0.3	0.3	0.3
Crushed flaxseed	0.0	0.5	1.0
Specific mineral mixture	1.0	1.0	1.0
Chemical composition (analyzed)			
Moisture (%)	9.37	9.55	9.18
Crude protein (%)	20.61	20.65	20.73
Crude fiber (%)	5.14	5.19	5.22
Ether extract (%)	5.11	5.11	5.23
Total ash (%)	6.06	6.08	6.11
ME (Kcal/kg)	3285	3290	3294
Calcium (Ca, %)	1.22	1.27	1.35
Phosphorus (P, %)	0.74	0.81	0.83
Salt (NaCl, %)	0.62	0.62	0.62
Vitamin A (IU/kg)	2497	2689	2644
Vitamin E (IU/kg)	38.17	38.42	38.38
Lysine (mg/100g)	0.83	0.90	0.96
Methionine (mg/100 g)	0.47	0.53	0.58
Potassium (K, %)	0.36	0.41	0.34
Aflatoxin B1 (μg/kg)	BDL	BDL	BDL
Aflatoxin B2 (μg/kg)	BDL	BDL	BDL
Aflatoxin G1 (μg/kg)	BDL	BDL	BDL
Aflatoxin G2 (μg/kg)	BDL	BDL	BDL
C18:1 Oleic acid (per 100 g fat)	1.09	1.16	1.20
C18:2 Linoleic acid (per 100 g fat)	0.18	0.40	0.43
C18:3 Linolenic acid (per 100 g fat)	0.07	0.39	0.45
C20:4 Arachidonic acid (per 100 g fat)	0.86	0.91	1.05
C20:5 Cis-5,8,11,14,17-Eicosapentanoic acid (per 100 g fat)	0.04	0.07	0.07
C22:6 Cis-4,7,10,13,16,19-Dicosahexanoic acid (per 100 g fat)	0.02	0.05	0.05

FS: Flaxseed supplementation; BDL: Below detection limit.

Linked Immunosorbent Assay kits from Bioassay Technology Laboratory, Shanghai, China (Cat. No. E0293Po-Progesterone, Cat. No. E0173Po-Estradiol-17β, Cat. No. E0284Po-IGF-1 and Cat. No. E0616Po-NEFA, respectively), following the manufacturer's protocol. All kits for endocrine and NEFA profiles presented intra- and inter-assay coefficients of variations <8% and <10%, respectively and the minimum detection limit of the assay for progesterone, estradiol and IGF-1 was 0.24 ng/mL, 2.2 pg/mL, 0.63 ng/mL and 1.17 μmol/L, respectively.

2.4. Body weight and feed intake measurements

Using a digital scale, body weight of each sow and individual birth weight of all piglets born were measured. Daily feed intake per sow was calculated by dividing the total feed intake in a day by the number of sows in a group. Body weight gain per sow was calculated as the difference between the weight of sow on succeeding day and the sow weight on preceding day and then divided the weight gain in a period (fortnightly) by the number of days in that period.

2.5. Subsequent reproductive and productive performance of sows

Data collected after weaning included wean to estrus interval, duration of estrus, wean to conception interval, number of pregnant sows at day 25 of gestation, number of sows that maintained pregnancy throughout gestation and number of sows that farrowed in the subsequent production cycle. The percentage of sows detected as being pregnant on day 25 post-breeding and the percentage of sows that farrowed in the subsequent production cycle were recorded relative to the number of sows weaned and number of sows bred. Litter performance in subsequent cycle included total piglets born at birth, piglets born alive, proportion of still births and mummified piglets and piglet mortality.

The diagnosis of pregnancy was performed at day 25 post-breeding with ultrasound machine (BestScan S6 Touch Digital Ultrasound Diagnostic System, BMV Technology Co., Ltd., Shenzhen, China) using a B-mode linear array abdominal transducer with 5/7.5 MHz interchangeable frequency.

2.6. Feed analysis

Feed samples within a group (control and flaxseed) used in the trial were collected at the end of study for analysis of moisture, crude protein, crude fat, crude fiber, acid insoluble ash, calcium, total phosphorus, common salt, aflatoxins total, vitamin A, vitamin E, fatty acids, lysine, methionine, potassium, magnesium and energy. The composition, quality and per-oxidation status of the ingredients (Table 1) were determined in representative samples by a Bureau of Indian Standards approved government laboratory (Punjab Biotechnology Incubator, Mohali, Punjab). The analyses included fatty acid profile (method 963.22), total fatty acids (method 972.28), free fatty acids (method 940.28), unsaponifiable matter (method 933.08), moisture (method ca 2a-45), insoluble impurities (method ca 3-46) and peroxide value (method 965.33) under the active oxygen method (method cd 12-57).

2.7. Statistical analysis

Data were analyzed according to a randomized complete block design by using MIXED model equation methods with SAS (statistical analysis system, version 9.3, USA) program. A mixed linear model, including the control diet, was fitted to the data to investigate the effects of supplemental flaxseed. The effects of treatments on endocrine milieu, metabolic profile, antioxidant status, body weight and feed intake variables were evaluated by repeated measures analysis of variance, with the effect of individual sows kept within the period. Due to lack of normality, the endocrine and metabolic data were transformed to alogarithmic scale. Subsequent reproduction (pregnancy rate, farrowing rate and proportion of pregnant sows) and production data were analyzed by using mixed linear and non-linear models according to the data distribution. Wean to estrus interval, wean to conception interval and subsequent litter size were normalized by using a log transformation prior to analysis to stabilize the variance. Tukey-Kramer adjustment was used for multiple comparisons of differences among all dietary treatments, including the control diet. All data presented were expressed as least squares means with their standard deviation (SD). For all analyses, a confidence level of $P<0.05$ was considered to be significant.

2.8. Ethics statement

This study was approved by the Institutional Animal Ethics Committee of Guru Angad Dev Veterinary and Animal Sciences University (Grant No. GADVASU/2019/IAEC/50/01).

3. Results

3.1. Metabolic profile

Flaxseed supplementation led to decrease in plasma cholesterol in the sows of group 2 from days 7-28 of the lactation period compared to those of groups 1 and 3 ($P<0.05$). A decreasing trend was also observed in plasma triglyceride levels in flaxseed supplemented sows (days 0-28 and days 0-14 of lactation period in group 2 and group 3 respectively, days and days 30-90 of gestation period in group 2 and group 3) compared to the control ones. The triglyceride levels declined more rapidly after day 21 until day 28 of lactation period and days 30-90 of gestation period in sows of groups 2 and 3 than those in group 1 ($P<0.05$). Other plasma metabolites (glucose, total protein, calcium, ALT, AST, GGT, BUN and creatinine) were similar in all the groups throughout the lactation and gestation period ($P>0.05$) (Figure 1).

3.2. Plasma endocrine profiles

The average plasma progesterone, estradiol-17β and IGF-1 concentrations in the three groups are given in Tables 2-4, respectively. There was no treatment effect on circulating progesterone concentrations of sows at any of the days measured during lactation period ($P>0.05$; days 0-28) and during the three sampling days covering early (day 30), mid (day 60) and late gestation (day 90) ($P>0.05$) (Table 2). Supplementation of flaxseed to sows failed to influence plasma estradiol-17β throughout the lactation period and at day 30 and day 60 of gestation as revealed by absence of difference in the hormone concentrations in all the groups ($P>0.05$). However, on day 90 of gestation period, plasma estradiol-17β levels were elevated in sows of group 2 than those in groups 1 and 3 ($P<0.05$) (Table 3). There was a significant treatment effect on average plasma IGF-I both in late lactation (day 28) as well as in early gestation (day 30) ($P<0.05$) when average plasma IGF-I was higher in group 3 than that in groups 1 and 2 ($P<0.05$). Similarly, increased IGF-1 values were also noticed in group 2 compared to group 1 at day 28 of lactation period and at day 30 of gestation period ($P<0.05$). Alternatively, no significant changes in plasma IGF-1 were noticed in all the groups at day 60 and 90 ($P>0.05$), suggesting that flaxseed failed to impact IGF-1 during mid and late gestation in the current experiment (Table 4).

3.3. Plasma NEFA

The effect of flaxseed supplementation on plasma NEFA was observed at day 28 of lactation when levels of NEFA were higher in sows of groups 2 and 3 compared to those fed control diets ($P<0.05$). Further, the levels were higher in sows of group 2 than those in group 3 at day 28 of lactation period, but there was no significant different. There was no effect of treatment on plasma NEFA on any of the days of gestation period as reveled by absence of difference in plasma NEFA ($P>0.05$) (Figure 2).

3.4. Body composition variables

An overall sow bodyweight change of (-4.2±0.4) kg with -4.6 kg, -4.3 kg and -3.8 kg in group 1, group 2 and group 3, respectively

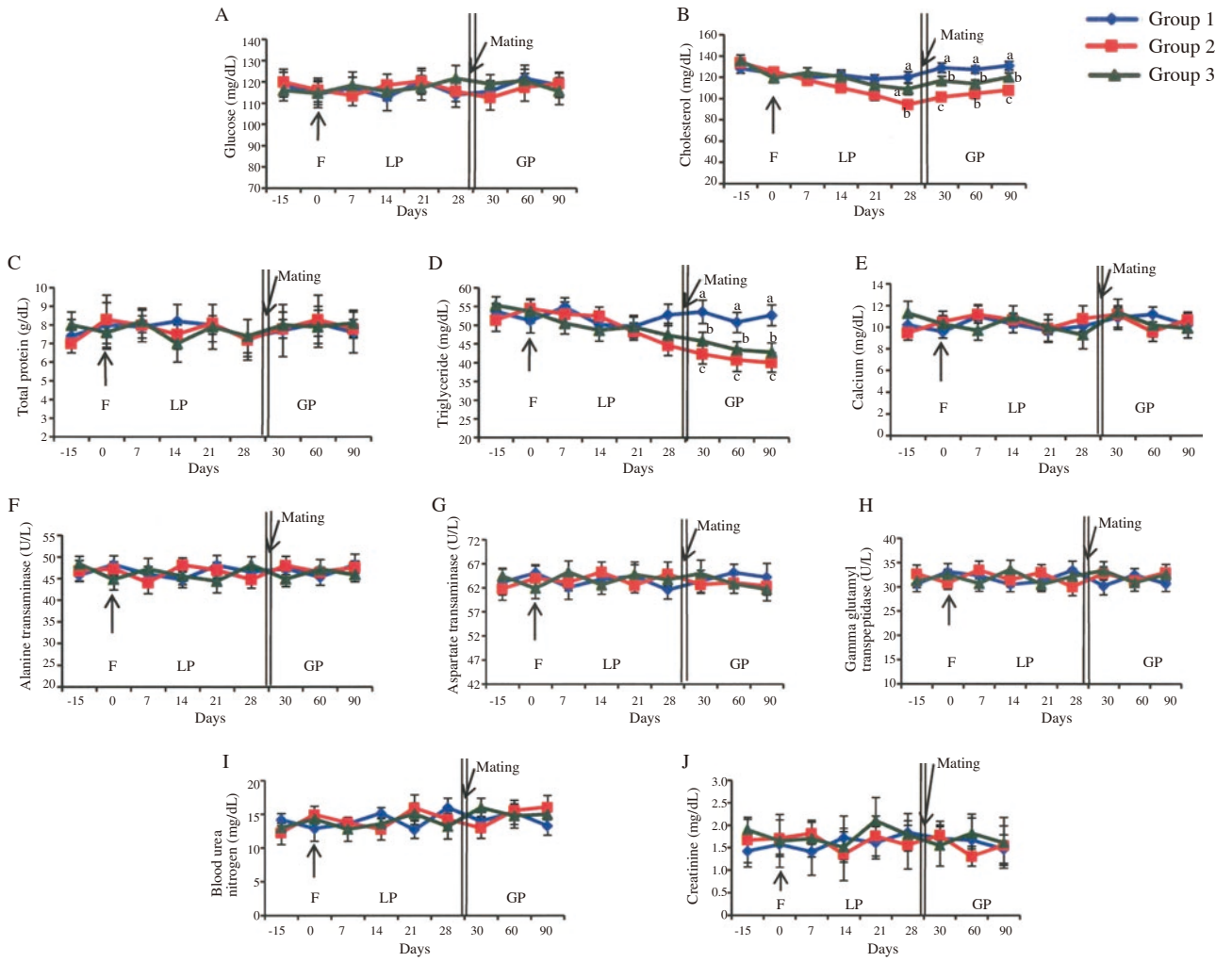

Figure 1. Plasma glucose (A), cholesterol (B), total protein (C), triglyceride (D), calcium (E), alanine transaminase (F), aspartate transaminase (G), gamma glutamyl transpeptidase (H), blood urea nitrogen (I), and creatinine (J) concentrations during the lactation and gestation period in sows. Data are expressed as mean±SD; n=7 in each group. Values with different alphabetic superscripts (a,b, c) differ significantly (P<0.05) from corresponding values in the three groups. Tukey-Kramer adjustment is used for multiple comparisons of differences among all dietary treatments. Group 1 serves as the control group, group 2 recieves 0.5% flaxseed supplementation, and group 3 receives 1.0% flaxseed supplementation. F: Farrowing; LP: Lactation period; GP: Gestation period.

was noticed during the lactation (days 0-28) (Table 5). Over the three sampling days in lactation period (day 0, day 14 and day 28), dietary flaxseed regimen showed no effect on body weight. The sows consumed (6.32±0.15) kg/day diet during 28-day lactation. Average sow feed intake in all the groups was almost similar (6.41 kg in group 1, 6.34 kg in group 2 and 6.44 kg in group 3, P>0.05) throughout the lactation period (days 0-28) and was not affected by treatment (P>0.05) (Table 5).

3.5. Subsequent reproductive and productive performance of sows

Supplementation of flaxseed during lactation had different impacts on the subsequent reproduction of sows (Table 6). Data indicated that 95.2% (20) sows exhibited estrus and were bred within 7 days post-weaning. Percentage of sows which exhibited estrus at breeding was 100.0% in group 1 and group 2 and 85.7% in group 3. One sow in group 3 did not exhibit estrus while one sow in group 1 did not conceive. In addition, one sow each in group 1, group 2 and group 3 had pre-mature delivery at day 32, 27 and 34 of pregnancy, respectively. No difference was observed in the duration of estrus at breeding, wean to conception interval and gestation length in all groups (P>0.05). Wean to estrus interval was longer in sows of group 3 followed by group 2 and group 1, but there were no significant differences (P>0.05). The proportion of sows pregnant on day 25 post-mating relative to the total sows in the study was marginally higher in group 2 than that in groups 1 and 3 and was not impacted by supplemental flaxseed (P>0.05). However, supplementation of flaxseed tended to increase the percentage of pregnant sows relative to sows bred (an increase of 14.3%) in groups 2 and 3 compared to their control counterparts (P>0.05). All in all, 16 sows farrowed with 5, 6 and 5 sows in group 1, group 2 and group 3, respectively.

Table 2. Plasma progesterone concentrations in sows in distinct feeding periods (ng/mL).

Period of production cycle	Group 1	Group 2	Group 3
Before farrowing (Day-15)	22.44±0.54	22.02±0.62	20.89±0.58
Farrowing (Day 0)	4.21±0.29	3.92±0.40	4.37±0.36
Lactation (Day 7)	1.46±0.20	1.73±0.32	1.64±0.17
Lactation (Day 14)	1.83±0.31	1.61±0.20	1.75±0.28
Lactation (Day 21)	1.78±0.20	1.86±0.22	1.58±0.24
Lactation (Day 28)	1.53±0.22	1.39±0.37	1.65±0.40
Gestation (Day 30)	18.04±1.07	18.57±0.86	18.49±1.08
Gestation (Day 60)	15.87±1.19	17.43±1.06	16.13±1.19
Gestation (Day 90)	17.34±1.24	18.74±1.06	17.05±1.25

Data are expressed as mean±SD; n=7 in each group. Analysis of variance is used in the analysis of progesterone. Group 1 serves as the control group, group 2 recieves 0.5% flaxseed supplementation, and group 3 receives 1.0% flaxseed supplementation.

Table 3. Plasma estradiol-17β concentrations in sows in distinct feeding periods (pg/mL).

Period of production cycle	Group 1	Group 2	Group 3
Before farrowing (Day-15)	204.68±2.09	200.42±2.55	198.52±2.02
Farrowing (Day 0)	42.85±1.05	43.88±1.04	45.02±1.0
Lactation (Day 7)	8.82±0.36	9.69±0.47	9.32±0.37
Lactation (Day 14)	9.27±0.29	9.10±0.32	8.92±0.32
Lactation (Day 21)	9.46±0.26	8.88±0.35	10.07±0.25
Lactation (Day 28)	9.41±0.34	10.13±0.28	9.75±0.22
Gestation (Day 30)	10.29±0.41	10.11±0.40	10.55±0.43
Gestation (Day 60)	9.73±0.28	10.27±0.36	10.22±0.35
Gestation (Day 90)	76.39±2.57$^{\#}$	119.19±2.68$^{\wedge}$	84.28±2.73*

Data are expressed as mean±SD; n=7 in each group. Analysis of variance is used in the analysis of estradiol-17β. The symbols #,^,* in the row differ significantly at P<0.05 (P=0.019 on day 90).

Table 4. Plasma IGF-1 concentrations in sows in distinct feeding periods (ng/mL).

Period of production cycle	Group 1	Group 2	Group 3
Before farrowing (Day-15)	157.5±1.7	155.7±2.2	153.6±2.1
Farrowing (Day 0)	155.8±2.2	152.9±2.0	154.4±2.0
Lactation (Day 7)	154.8±2.4	157.9±2.3	156.3±2.2
Lactation (Day 14)	158.9±2.3	153.6±2.6	157.7±2.5
Lactation (Day 21)	155.4±2.3	154.8±2.1	154.2±2.3
Lactation (Day 28)	161.7±2.3$^{\#}$	167.6±2.2$^{\wedge}$	172.8±2.2*
Gestation (Day 30)	177.1±2.0$^{\#}$	198.9±2.2$^{\wedge}$	211.2±2.5*
Gestation (Day 60)	153.9±2.3	153.7±2.1	156.3±2.5
Gestation (Day 90)	157.5±2.4	155.7±2.7	158.1±2.0

Data are expressed as mean±SD; n=7 in each group. Analysis of variance is used in the analysis of insulin like growth factor-1 (IGF-1). The symbols #,^,* in the row differ significantly at P<0.05 (P=0.014 on day 28, P=0.023 on day 30).

Table 5. Body weight and feed intake in sows in distinct feeding periods.

Period of production cycle	Group 1	Group 2	Group 3
Body weight (kg/sow)			
Before farrowing (Day-15)	175.3±3.7	172.8±3.9	177.2±2.8
Farrowing (Day 0)	160.3±2.6	158.4±3.2	160.9±3.4
Lactation (Day 14)	158.5±3.3	157.3±2.7	159.6±2.9
Lactation (Day 28)	155.7±2.8	154.1±2.4	157.1±2.5
Feed intake (kg/day)			
Farrowing (Day 0)	6.41±0.49	6.34±0.86	6.23±0.31
Lactation (Day 14)	6.33±0.72	6.29±0.29	6.16±0.63
Lactation (Day 28)	6.44±0.54	6.33±0.47	6.28±0.78

Data are expressed as mean±SD; n=7 in each group. Analysis of variance is used in the analysis of body weight and feed intake. Linear effect is used for supplemental flaxseed. Tukey-Kramer adjustment is used for multiple comparisons of differences among all dietary treatments.

The average litter size at birth, stillborn and mummified rate, piglet weight at birth and piglet mortality of subsequent parity is shown in Table 6. Sows fed flaxseed during gestation exhibited a dose effect of treatment on the piglets born alive which was maximum in group 3 followed by group 2 and group 1 in the subsequent farrowing, while reverse was true for stillborn and mummified rate (P<0.05). Piglet mortality was lower in groups 2 and 3 compared to group 1, but there was no significant difference (P>0.05). No effect of flaxseed supplementation was observed on total number of piglets born and piglet weight (P>0.05) (Table 6).

Table 6. Reproductive and productive performance of sows in different groups in subsequent farrowing.

Parameters	Group 1	Group 2	Group 3
Number of sows in the study (*n*)	7	7	7
Number of sows that exhibited oestrus at breeding (*n*)	7	7	6
Duration of standing oestrus (h)	49.7±1.5	51.4±1.6	50.0±1.7
Weaning to oestrus interval (h)	108.0±3.9	111.4±2.7	114.0±3.8
Number of sows bred (*n*)	7	7	6
Weaning to conception interval (h)	156.0±2.8	157.7±2.4	156.3±3.3
Number of sows pregnant at day 25 post-breeding (*n*)	6	7	6
Number of sows farrowed (*n*)	5	6	5
Pregnancy rate relative to sows in study (%)	85.7	100.0	85.7
Pregnancy rate relative to sows bred (%)	85.7	100.0	100.0
Number of farrowing relative to sows in study (*n*)	5/7 (71.4%)	6/7 (85.7%)	5/7 (71.4%)
Number of farrowing relative to sows bred (*n*)	5/7 (71.4%)	6/7 (85.7%)	5/6 (83.3%)
Gestation length (days)	115.6±0.5	115.7±0.7	116.2±0.4
Total piglets born at birth (*n*)	12.2±0.7	12.5±0.9	12.4±0.4
Piglets born alive (*n*)	10.0±0.6	10.8±0.8	11.6±0.5
Stillbirths and mummified piglets (%)	16.4±1.5[a]	13.0±1.4[b]	6.7±1.1[c]
Piglet mortality (%)	6.3±1.4	4.2±1.0	4.0±1.3
Piglet weight at birth (kg)	1.22±0.20	1.21±0.18	1.24±0.16

Data are expressed as mean±SD; *n*=7 in each group. Reproduction and production data are analyzed by using mixed linear and non-linear models. Values with different alphabetic superscripts (a, b, c) differ significantly at $P<0.05$ from corresponding values in a row.

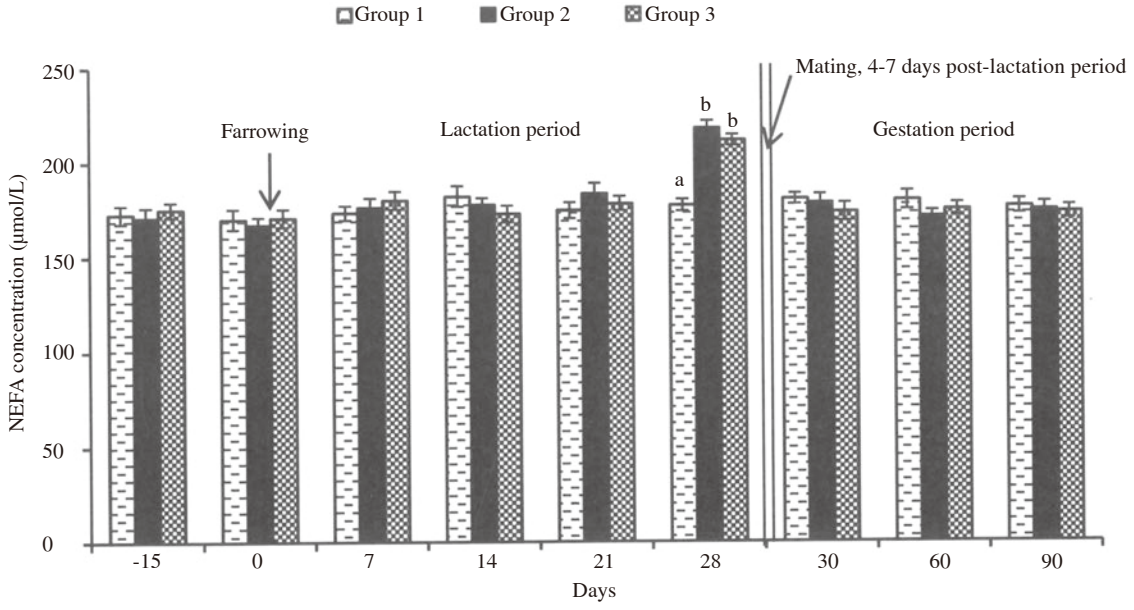

Figure 2. Plasma non-esterified fatty acid (NEFA) concentrations in sows in distinct feeding periods. Values with different alphabetic superscripts (a, b) differ significantly ($P<0.05$) from corresponding values in the three groups. Tukey-Kramer adjustment is used for multiple comparisons of differences among all dietary treatments.

4. Discussion

The present study is a comprehensive approach to advocate the usefulness of flaxseed supplementation in lactating sows throughout the lactation and gestation period. The sows of group 2 and group 3 exhibited a decrease in plasma concentrations of cholesterol during lactation period. A consistent decrease in triglycerides throughout the lactation and gestation period was observed in the sows of group 2 subsequent to flaxseed supplementation compared to their contemporary mates. However, these values remained within the physiological range of plasma cholesterol [(98.99-134.67) mg/dL[20] and plasma triglycerides [(0-63.71) mg/dL][21] in sows. Dietary supplementation of n-3 PUFA can lead to down-regulation of fatty acid synthesis gene expression (cholesterol regulatory element binding protein-1c) and up-regulation of gene expression involved in fatty acid oxidation (peroxisome proliferator-activated receptor α)[12]. Moreover, feeding diets rich in n-3 PUFA leads to decreased hepatic fatty acid synthesis, reduces activity of triglyceride-synthesizing enzymes (diacylgylcerol acyltranferase and/or phosphatidic acid phosphohydrolase) and subsequently leads to decreased triglyceride

levels[11]. Previous studies[22] in prepubertal gilts supplemented with fish oil containing n-3 PUFA starting at the age of 120 days and continuing upto 45 days thereafter also showed reductions in cholesterol and triglyceride levels and observed positive relationship with increased antioxidant activity. Plasma glucose and total protein recorded in the present study revealed no adverse impact of flaxseed supplementation on sow. These metabolites were also not altered following supplementation of diet of sows with flaxseed[23]. Previous studies in sow are lacking to substantiate the present findings of plasma concentrations of calcium, AST, ALT and GGT, BUN and creatinine with respect to the flaxseed supplementation.

In the present study, dietary flaxseed regimen showed no effect on plasma progesterone during lactation and gestation period. Similarly, no alteration in plasma progesterone during the gestation period was observed in sows subsequent to dietary supplementation with different forms of flaxseed (extruded flaxseed, 10.0% dry matter; flaxseed meal, 6.5% dry matter; flaxseed oil, 3.5% dry matter) from day 68 of gestation until day 21 of lactation[23]. Feeding gilts with PUFA rich diet resulted in 3.5-fold higher ovarian derived progesterone compared to peripheral progesterone which gives a more legitimate picture, owing to the site of progesterone production and synthesis, higher binding capacity to uterus through modification of receptors, pulsatility nature and confined action of hormone[24]. In contrast, an overall reduction in progesterone concentrations was observed during late gestation and early lactation in sows fed upto five percent flaxseed[25].

Supplementation of flaxseed to sows in the present study had no impact on plasma estradiol-17β throughout lactation and at days 30-60 of gestation period. Our findings regarding estradiol concentrations are in line with those reported by Farmer *et al*[23] that circulating concentrations of estradiol remained unaltered in mid gestation (day 62) and lactation (day 2 and day 21), subsequent to dietary flaxseed supplementation from day 68 of gestation until day 21 of lactation. However, supplementation of flaxseed exhibited positive impact on plasma estradiol-17β at day 90 in sows of group 2 compared to sows of group 3 and group 1. Eventually, in the present study a higher proportion of sows that maintained pregnancy throughout the gestation in group 2 (85.6%) than in group 3 (71.4%) and group 1 (71.4%) might have contributed toward their elevated plasma estradiol-17β at day 90 since the fetoplacental unit is the major source of estrogen production in sows. Increased estradiol from the developing conceptus is necessary for reduction in uterine epithelial prostaglandin so that successful implantation and a continued secretion of progesterone can occur during pregnancy[26].

Positive effect of flaxseed supplementation on plasma IGF-I was observed which is in agreement with the findings of Li *et al*[27], who also reported higher IGF-1 at day 28 following supplementation of n-3 PUFA diet. Feeding flaxseed during lactation facilitates the sows to restore their lactation body weight losses observed during late lactation and early gestation thereby resulting higher IGF-1. Furthermore, after weaning and until day 35 post-breeding, sow quickly changes from a catabolic to an anabolic state and is indicated by the rise of IGF-1 during the said period[28]. However, flaxseed supplementation failed to impact plasma IGF-1 during mid (day 60) and late (day 90) gestation in the current experiment. Previous studies[29] have also demonstrated that dietary supplementation of sows with flaxseed from day 90 of gestation until weaning (24 to 28 days post-farrowing) failed to influence plasma IGF-1 in the late gestation (day 105).

The effects of flaxseed on the plasma NEFA are consistent with the observations of other studies[30] which demonstrated that supplementation of linseed to sow diet in late gestation and throughout lactation failed to have any impact on plasma NEFA. Similarly, Eastwood *et al*[31] also observed that supplementation of sows with PUFA rich fish oil at 110 days of gestation and continuing until weaning at days 26±2 (after farrowing) did not have any effect on plasma NEFA until day 21 of lactation. The authors recorded elevated plasma NEFA toward late lactation (day 26). Long-term dietary PUFA may lead to changes in fatty acid esterification, as evidenced by increased NEFA concentrations in late lactation in flaxseed supplemented sows, indicating these sows may have been mobilizing more body fat[31,32]. However, a recent study on lactating sows supplemented with fish oil (2.5%) from gestation period (day 84) until day 16 of lactation exhibited greater impact on milk NEFA profile compared to plasma NEFA profile[33]. In fact, analysis of milk NEFA profile may have been a more desirable option for delineating the alterations in NEFA of flaxseed supplemented sows in the current study.

In the present study, sow body weight and feed intake were not affected by flaxseed supplementation during lactation. Numerous studies[31,34,22,35] have also shown no alterations in body weight and feed intake following supplementation of PUFA to sows during lactation. The suggested reason was flaxseed supplementation in lactation diet of sows tended to increase caloric intake to support growth and development of the nursing litter without any influence on body weight as observed in the current study. Moreover, flaxseed used in the current study was manufactured in relatively small batches to reduce the length of storage time. Long-term storage of flaxseed may reduce feed intake, nutrient absorption and affect animal performance[36].

The investigation of flaxseed on reproduction is more straight-forward in monogastrics than in ruminants since pigs have a simple stomach and microbial modification of fatty acids is insignificant. Limited studies in pigs have shown that supplementation α-linolenic acid in the form of flaxseed is effective in improving their reproductive performance (increased pregnancy rate by 4.3 percent and farrowing rate by 9.5 percent)[35]. Although proportion of sows pregnant relative to total number of sows does not

appear to be affected by supplemental flaxseed, which may partially be due to the small number of animals, percentage of sows pregnant and sows farrowed relative to sows bred are definitely improved in the present study. Moreover, flaxseed benefits sow fertility through different mechanisms and concentrations[37]. In the present study, subsequent reproduction response was determined by using multiple criteria *viz*. percentage of sows exhibiting estrus, wean to estrus interval, pregnancy rate and farrowing rate. A concentration of 0.5% flaxseed was the most effective dietary treatment in achieving the greater number of sows exhibiting estrus, higher proportion of sows pregnant relative to sows bred and retention of pregnancy but it did not appear to influence wean to estrus interval.

Similar to our findings, supplementation of flaxseed to gestation diets failed to affect total litter size and piglet weight at birth in numerous studies[9,38,35,33]. However, impact of flaxseed supplementation on piglets born alive and piglet survivability observed in this study is in agreement with the findings of previous studies[34,39,40] which demonstrated that the potential benefits of flaxseed on growing fetus include enhanced neural development, better immune response and improved near term piglet survival through increased concentrations of immunoglobulin G in colostrum and milk and subsequently increased the transfer of antibodies and leukocytes to the developing piglets.

The major limitation in the present study was usage of small number of animals.

In conclusion, flaxseed supplementation at a rate of 0.5 percent of the dry matter starting on day 1 of lactation until the day of subsequent farrowing is able to improve endocrine profiles, NEFA concentrations and body composition variables and subsequent reproductive performance of sows.

Conflict of interest statement

The authors declare that there is no conflict of interest that would prejudice the impartiality in this experiment.

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Authors' contributions

Sukhjinder Kaur, Ashwani Kumar Singh and Ajeet Kumar were involved in planning and execution of research work, data recording and manuscript writing. Ashwani Kumar Singh performed statistical analysis. Mrigank Honparkhe, Prahlad Singh and Udeybir Singh helped in various parametric estimations. Udeybir Singh also performed partial feed analysis and administration of treatment.

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