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Effect of Dietary Enrichment with Omega 3 and 6 Fatty Acids on Blood Metabolites, Hormone Concentration and Ovarian Function in Sahiwal Heifers

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ABSTRACT

Background: Supplementation of fat, especially those having unsaturated fatty acids has been proposed to carry positive effects on the reproductive organ, beside providing dietary energy to the animals. This experiment was designed to examine the effects of dietary ω-3 or ω-6 fatty acid (FA) rich oil supplementation on blood FA, metabolite and hormone concentrations; ovarian follicular growth and corpus luteum (CL) size in Sahiwal breed heifers.

Methods: Eighteen heifers of 18.33±1.14 months of age and 194±4.16 kg of mean body weight were randomly assigned to 3 diets and individually fed as per ICAR (2013) diets. The diets include chopped wheat straw, green fodder and concentrate mixture containing either (i) no added PUFA rich oil but palm oil @ 3.5% (PO; n=6); (ii) 3.5% added soybean oil as ω-6 FA source (SO; n=6); or (iii) 3.5% added linseed oil as ω-3 FA source (LO; n=6).

Result: SO increased (P<0.05) the plasma concentration of ω-6 FA while LO increased (P<0.05) the plasma ω-3 FA. Plasma glucose, triglyceride and non-esterified fatty acid (NEFA) concentrations was not affected due to different diets. Plasma total cholesterol and HDL-cholesterol were significantly higher (P<0.05) in SO and LO in comparison to PO. However, LDL-cholesterol was at par among all the treatments. Growth Hormone (GH) was not influenced due to different types of oil in heifers' concentrate mixture. Insulin concentration increased (P<0.05) in LO compared to others. IGF-1 was statistically higher (P<0.05) in SO and LO as compared to CON, which among themselves also varied significantly. Plasma progesterone concentration at day 12 post estrous was higher (P<0.05) in LO. PUFA rich oil supplementation in the concentrate mixture of heifers (SO and LO) increased (P<0.05) the size of the ovulatory follicles as well as size of CL. It was inferred that feeding PUFA rich oil to pre-pubertal Sahiwal heifer's results in advantageous changes in the blood metabolites, plasma hormones and ovarian functions.

Key words: Hormones, Linseed oil, Metabolites, Ovarian functions, Sahiwal heifers, Soybean oil.

INTRODUCTION

Replacement of older animals with female young stock is the only way for sustained milk production. For maximum output from a commercial dairy farming, sound rearing approach of heifers so as to replace the older and unproductive animals through the culling play significant role. However, heifer production is considered to be the most expensive part of dairy farm operation because it needs more inputs for a longer period with no immediate returns. Proper planning for replacement heifers affects the success and longevity of a dairy herd. A heifer must attain puberty by 15 months of age to optimize production. Adequate nutrient supply to the animal is paramount to development of heifers. Age at puberty is an important production trait and hormones, nutrition and genotype play key roles in the attainment of puberty (Bellows and Hall, 1996).

Supplementation of fat with varying degree of unsaturation, beside providing energy to animals, has a positive effect on the reproductive aspects such as the establishment of puberty, semen production (Castellano *et al.*, 2010), maternal recognition of pregnancy, follicle development, quality of oocytes and modification in the mechanism of synthesis and secretion of hormones involved in reproductive processes. Polyunsaturated fatty acids (PUFA) such as linoleic acid, alpha-linolenic acid, eicosapentaenoic acid and docosahexaenoic acid may target reproductive tissues and alter reproductive function and fertility in cattle (Thatcher *et al.*, 2004). Plant oils with

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predominantly polyunsaturated fatty acids have also been shown to increase serum concentrations of lipoprotein cholesterol, growth hormone (GH) and insulin, to elevate intra-follicular concentrations of high-density lipoprotein cholesterol (HDL-cholesterol) and to enhance the production of IGF-I by luteal tissue *in vitro* (Ryan *et al*., 1995). Some studies (Thomas *et al*., 1997) has reported that dietary supplemental soybean oil increased the serum concentrations of insulin and GH as well as follicular IGF-I concentrations, which might positively impact reproduction by stimulating granulosa cell proliferation in ovary (Webb *et al*., 1992). Similarly, other study (Ryan *et al*., 1992) confirms that feeding cow supplemental dietary fat with varying saturation increased serum progesterone and cholesterol concentrations. Circulating cholesterol acts as precursor for luteal progesterone synthesis (Staples *et al*., 1998). Polyunsaturated fat stimulates a marked increase in serum insulin with a greater rate of ovarian follicular growth in cattle (Thomas *et al*., 1997) compared to saturated fats. Dietary ω-3 and ω-6 rich oil supplements leads to greater blood cholesterol concentration and increased number of small and total follicles in early lactating cows (Gandra *et al*. 2017). Blood cholesterol concentration has been reported to be positively associated with reproductive performance (Westwood *et al*., 2002) including the expression of oestrus and conception rate in dairy cows. Sahiwal is one of the best milch breeds in the tropics (Ilatsia *et al*., 2011) and nutritional intervention with essential fatty acids may further improve their reproductive functions. Therefore, the current study was planned to investigate the role of dietary ω-3 or ω-6 FA rich oil supplementation in improving metabolite and hormone status; ovarian follicular and corpus luteal attributes in Sahiwal heifers.

MATERIALS AND METHODS

The experiment was performed after due permission from the Institutional Animal Ethics Committee, CPCSEA, New Delhi.

Experimental animals and diets

The experiment was conducted at cattle farm, Department of Animal Genetics and Breeding, LUVAS, Hisar, using 18 sahiwal heifers with no overt clinical sickness. The cows were stratified at random into 3 groups. Within group the heifers were assigned to one of the three iso-nitrogenous and iso-energetic diets containing weighed quantity of green fodder and concentrate mixture for a period of five months to meet out the nutrient requirement as per ICAR (2013). Wheat straw was fed *ad libitum*. The concentrate mixture in three dietary treatments contained: (1) no PUFA rich oil but Palm oil as control (PO); (2) 3.5% soybean oil as ω-6 PUFA source (SO) and (3) 3.5% linseed oil as ω-3 PUFA source (LO). Palm oil was added in the control diet to make different rations iso-caloric. The diets were fed twice daily at 8:00 AM and 4:00 PM. Animals were dewormed against internal parasites and sprayed with ecto-parasiticidal solution before start of the experiment adopting standard protocol. Routine protection against hemorrhagic septicemia and foot and mouth disease was done by immunization with vaccines. All experimental animals were housed in feeding stall having arrangements for individual feeding and watering. The animals under different dietary treatments were maintained under isomanagerial conditions and similar husbandry practices except the different feeding treatments.

Chemical analysis of feed ingredients and different oils

The chemical compositions of feed ingredients and the different concentrate mixtures after adding respective oils were analyzed according to methods of the official methods of analysis of AOAC (2005). The fatty acid composition of different oil sources was estimated by chromatographic method following the procedure proposed by Folch *et al*. (1957) and Park and Goins (1994).

Collection and analysis of blood samples

Blood samples from these 18 sahiwal heifers were collected at monthly intervals to determine the concentration of plasma fatty acids; metabolites like, glucose, triglycerides, non-esterified fatty acid, cholesterol, HDL, LDL and plasma hormones *viz.* growth hormone, insulin and IGF-1. Additional blood samples were collected on day 12 post estrous in each heifer to estimate plasma progesterone concentration. All blood samples (7 to 10 ml) were collected by jugular veinpuncture into vials containing Ethylene Diamine Tetra Acetic acid (EDTA) and Sodium fluoride, chilled immediately after collection and centrifuged (1,500 × g, 15 min) within 2 h; plasma was harvested and stored at -20°C until further processing. The blood metabolites were measured using Ebra EM-200 biochemistry analyzer (Sr. No. B110318, Ebra Mannheim, Transasia Bio-medicals ltd). Plasma NEFA concentrations were analyzed by automated analyzer (kit 99075401; Wako Pure Chemical Industries, Osaka, Japan). Progesterone concentrations were analyzed by ELISA kits following the manufacturer's instructions (Calbiotec Inc., 1935, Cordell Ct, El Cajon, CA 92020, USA). GH, insulin and IGF-1 were measured by using respective ELISA kits following the manufacturer's instructions (Sunred Biological Technology Co., Ltd, Shanghai). Values of different parameters recorded at various intervals were pooled and represented as overall mean value for the experiment.

Ovarian ultrasonography

Cows were observed for estrous behaviortwice daily for 30 minutes to detect the animal in heat. Trans-rectal ovarian ultrasonography (7.5-MHz transducer; Sonoscape S6) examination was carried out on the day of estrous to assess the number of small (<5 mm), medium (5-9.9 mm) and large (≥10 mm) sized follicles on each ovary. The diameter of the ovulatory and subordinate follicle as the average length and width of the antrum was also determined. The diameter of subsequent CL and progesterone concentration on day 12 post-estrous were measured for individual animals.

Statistical analysis

The experimental heifers were divided randomly in to three dietary treatment groups (PO, SO, LO) of six animals each on the basis of body weight and age following factorial completely randomized design (FCRD). The heifers averaged 194±4.16 kg in body weight and 18.33±1.14 months in age. Data obtained were subjected to statistical analysis as per Snedecor and Cochran (1994) using factorial completely randomized design (CRD). The mean differences among different treatments were separated by Duncan's multiple range tests (Kramer, 1957). Consequently, a level of (P<0.05) was used as the criterion for statistical significance.

RESULTS AND DISCUSSION

The ingredient and chemical composition of different concentrate mixtures, wheat straw and maize fodder has been presented in Table 1. Ingredient composition of concentrate mixture of all the three dietary treatment groups was similar except for the type of the oil used. 3.63 kg each of palm oil, soybean oil and linseed oil were added to 100 kg concentrate mixture of PO, SO and LO, respectively, to make the final enrichment @ 3.5%. Palm oil was added in the control to make different diets iso-caloric. Similar crude protein in different diets shows that the different rations were iso-nitrogenous in nature.

Fatty acid composition of oils and plasma

SFA per cent was highest in palm oil (Table 2). MUFA content of palm oil, soybean oil and linseed oil was ranged from 18.18-39.14%. Linseed oil had the highest proportion of PUFA followed by soybean oil and least in palm oil. Among PUFA, ω-6 proportion was highest in soybean oil (52.30%) while ω-3 was found to be highest in linseed oil (55.60%).

PUFA supplementation had a profound effect (P<0.05) on lowering the plasma SFA level. MUFA concentration was not affected (P>0.05) by PUFA addition in the diet of heifers. Soybean and linseed oil supplemented group (SO and LO) had significantly (P<0.05) higher overall mean plasma PUFA concentration (22.97 and 22.88%, respectively) against to 18.94% (Table 3) in the control (PO), similar to as reported by previously (Tran *et al*., 2016; Khoshvaght *et al*., 2016). Soybean oil feeding (SO) caused significant increase (P<0.05) in plasma ω-6 concentration while linseed oil supplementation (LO) had significant effect (P<0.05) in amplifying the plasma ω-3 FA concentration compared to control.

Plasma metabolites and hormone concentrations

Diets supplemented with different PUFA oil showed no evident (P>0.05) effects on the mean plasma glucose of different groups (Table 4). In accordance with this, Whitney *et al*. (2000) and Childs *et al*. (2008b) also observed no effect of inclusion of ω-6 or ω-3 rich oil on plasma glucose in heifers. The serum triglyceride and NEFA concentration showed no response to the PUFA supplementation in heifers' diet. The experimental pooled values of cholesterol concentration in the serum of group LO (168.14 mg/100 ml) was significantly higher (P<0.05) than SO (147.92 mg/

Table 1: Ingredient and chemical composition analysis (%) of the concentrates and forage.

Attribute	Concentrate mixture			Roughages	
	PO	SO	LO	Wheat straw	Maize fodder
Maize	30	30	30	-	-
GNC	25	25	25	-	-
MC	10	10	10	-	-
WB	32	32	32	-	-
MM	2	2	2	-	-
Salt	1	1	1	-	-
PO, kg	3.63	-	-	-	-
SO, kg	-	3.63	-	-	-
LO, kg	-	-	3.63	-	-
			Chemical composition*		
DM	93.16	92.92	93.24	94.43	25.11
Organic matter	91.42	91.17	91.03	90.73	89.63
CP	21.25	20.98	21.14	2.45	10.45
Ether extract	5.32	5.18	5.57	2.87	4.31
Ash	8.58	8.83	8.97	9.27	10.37
Crude fibre	8.30	9.79	9.29	36.79	26.40
NDF	20.20	20.86	19.63	80.01	70.69
ADF	14.30	14.50	14.35	52.46	34.92

*Each value represents the mean of three observations on DM basis except for dry matter.

Abbreviations: GNC: Groundnut cake, MC: Mustard cake, WB: Wheat bran, MM: Mineral mixture, PO; Palm oil, SO: Soybean oil, LO: Linseed oil, DM: Dry matter, CP: Crude protein, CF: Crudefibre, ADF: Acid detergent fibre, ADL: Acid detergent lignin, NDF: Neutral detergent fibre.

100 ml), which further was higher (P<0.05) than that of PO (123.44 mg/100 ml). The results of study are corroborated with Childs *et al.* (2008a) who reported increased cholesterol concentrations in heifers fed PUFA supplemented compound feed. Gandra *et al.* (2017) supplemented lactating cows' diet with ω-3 and ω-6 rich oil and found greater blood cholesterol concentration. There was no effect of feeding ω-3 and ω-6 fatty acid rich oils on serum LDL-cholesterol level but, plasma HDL concentration was significantly (P<0.05) higher in heifers fed PUFA supplemented compound feed over control diet (Table 4). Similarly, Ghasemzadeh-Nava *et al.* (2011) observed increased plasma cholesterol and HDL concentration with no influence on LDL in Holstein cows supplemented with soybean and fish oil.

Table 2: Fatty acid composition of oils used in different diets (g/100 g FAME)*. n=3

Fatty acid group	Supplemental oils		
	Palm oil	Soybean oil	Linseed oil
SFA (%)	49.76	15.15	9.82
MUFA (%)	39.14	23.90	18.18
PUFA (%)	11.10	60.95	72.00
ω-6 FA (%)	10.78	52.30	16.40
ω-3 FA (%)	0.32	8.65	55.60
ω-3/ω-6	0.03	0.17	3.39
PUFA:SFA	0.22	4.02	7.33

*Each value is mean of three observations.

Abbreviations are: SFA: Saturated fatty acids from C8:0-C20:0, MUFA: Monounsaturated fatty acid from C18:1, PUFA: Polyunsaturated fatty acid from C18:2-C18:3.

Table 3: Effect of diets on fatty acid concentrations (g/100 g FAME) of plasma* (Means±S.E.).

Parameter	Treatments		
	PO	SO	LO
SFA	58.23^{a}±0.74	54.91^{b}±1.04	54.80^{b}±1.06
MUFA	22.83±0.77	22.13±1.32	22.32±1.02
PUFA	18.94^{b}±1.06	22.97^{a}±0.87	22.88^{a}±1.19
ω-6FA	17.42^{b}±1.05	21.51^{a}±0.83	18.23^{b}±1.03
ω-3 FA	1.57^{b}±0.10	1.46^{b}±0.11	4.65^{a}±0.26
ω-6/n-3	11.31^{a}±0.81	14.73^{a}±0.83	3.92^{b}±0.35

*Each value is mean of six replicates and every replicate having three observations. The mean values bearing different superscripts in a row differ significantly (P<0.05).

Abbreviations are: SFA: Saturated fatty acids from C8:0-C20:0, MUFA: Monounsaturated fatty acid from C18:1, PUFA: Polyunsaturated fatty acid from C18:2-C18:3.

In contrary to Thomas *et al.* (1997) and Whitney *et al.* (2000), the serum growth hormone did not respond (P>0.05) to the PUFA rich oil supplementation in our study. Overall mean insulin level was considerably (P<0.05) higher in linseed oil fed heifers as compared to others as also confirmed earlier by Thomas *et al.* (1997) who found marked increase in serum insulin in PUFA fed animals. Mean plasma IGF-1 was significantly (P<0.05) higher in linseed oil supplemented group than other groups and that of soybean oil supplemented group was significantly higher (P<0.05) than that of the control group. Thomas *et al.* (1997) also noted increased serum concentrations of IGF-I concentrations on dietary supplemental soybean oil. Len *et al.* (2016) fed PUFA feed source to buffalo calves and found significantly (P<0.05) increased plasma IGF-1. Garcia *et al.* (2003) also reported moderate increase in circulating concentrations of IGF-1 during pubertal development in cattle by feeding PUFA. Linseed oil supplementation increased (P<0.05) the mean plasma progesterone concentration (7.34 ng/ml) at day 12 post-estrous in contrast to the heifers of control and soybean oil fed groups (4.02 and 5.23 ng/ml, respectively). Dirandeh *et al.* (2014) observed that mean plasma progesterone concentration on day 15 of the synchronized cycle was higher in cows fed soybean oil and linseed oil than in those fed control.

Ovarian characteristics

Studies in the past has reported inconsistent finding regarding the effect of PUFA on small and medium sized

Table 4: The effect of diet on plasma metabolites and hormone concentrations* (Means±S.E.)

Parameter	Treatments		
	PO	SO	LO
Glucose, mg/dl	64.77±0.99	65.58±1.60	62.78±1.02
Triglyceride, mg/dl	19.70±0.85	20.81±0.97	20.23±0.51
NEFA, mmol/l	0.38±0.01	0.36±0.02	0.39±0.02
Cholesterol, mg/dl	123.44^{c}±2.59	147.92^{b}±5.56	168.14^{a}±6.08
HDL, mg/dl	80.85^{b}±2.13	113.13^{a}±4.31	122.66^{a}±5.75
LDL, mg/dl	22.37±0.63	22.60±0.75	22.55±0.58
GH, ng/ml	6.25±0.38	6.87±0.51	6.81±0.50
Insulin, ng/ml	0.152^{b}±0.01	0.168^{ab}±0.02	0.172^{a}±0.01
IGF-1, ng/ml	113.80^{c}±2.57	124.57^{b}±3.45	136.70^{a}±3.46
Progesterone, ng/ml	4.02^{b}±0.41	5.23^{b}±0.31	7.34^{a}±0.86

*Each value is mean of six replicates and every replicate having three observations.

The mean values bearing different superscripts in a row differ significantly (P<0.05).

Table 5: The effect of different diets on ovarian characteristics of heifers*.

Parameter	Treatments		
	PO	SO	LO
		No. of follicles	
Small (<5 mm)	5.33±0.80	3.33±0.56	4.00±0.78
Medium (5-9.9 mm)	2.00±0.37	2.67±0.49	2.83±0.48
Large (≥10 mm)	0.50^{b}±0.22	1.17^{a}±0.17	1.17^{a}±0.17
All follicles	7.83±0.40	7.17±0.30	8.00±0.73
		Size of follicles	
Ovulatory follicle (F_1), mm	9.32^{b}±0.47	11.88^{a}±0.92	12.87^{a}±0.88
Subordinate follicle (F_2), mm	6.17±0.48	6.83±0.54	7.50±0.62
F_1-F_2, mm	3.15±0.41	5.05±1.13	5.37±0.57
Size of CL, mm	11.40^{c}±0.46	13.28^{b}±0.26	14.68^{a}±0.60

*Each value is mean of six observations.

The mean values bearing different superscripts in a row differ significantly (P<0.05).

ovarian follicles. In the present study, linseed or soybean oil supplementation in the diet of heifers had no effect on the mean number of small (<5 mm) and medium (5-9.9 mm) sized ovarian follicles. But, mean number of large sized follicles (≥10 mm) increased significantly (P<0.05) upon PUFA rich oil supplementation (Table 5). Total number of all types of follicle was also not affected by the type of dietary fatty acids. In contrary to the results of current study, Gandra *et al*. (2017) found increased number of small and total ovarian follicles in early lactating cows supplemented with ω-3 and ω-6 rich oil. SO and LO increased the size of ovulatory follicle (F_1) significantly (P<0.05) as compared to PO. In previous findings of Dirandeh *et al*. (2013) and Bilby *et al*. (2006) reported that the number and size of developing follicles increased in cows fed linseed oil enriched in ω-3 PUFA. Similarly, Ghasemzadeh-Nava *et al*. (2011) reported that the size of the F_1 follicle was significantly (P<0.05) greater in cows that consumed a diet containing fish oil (ω-3) or soybean oil (ω-6). The size of subordinate follicle (F_2) and the difference F_1-F_2 remained largely unaffected by the diet. CL on day 12 of estrous cycle increased in size (P<0.05) in SO and LO as compared to PO. Many other studies in past also support the present findings of increased mean diameter of the ovulatory follicle (Ambrose *et al*., 2006; Mendoza *et al*.,2011) and CL (Petit *et al*., 2002) when dairy cows were fed diets high in ω-3 fatty acids.

CONCLUSION

The results of the present study inferred that beside providing energy for various metabolic functions, feeding omega-3 and omega-6 fatty acid rich oil prior to commencement of puberty in sahiwal heifers results in advantageous changes in the blood metabolites like plasma fatty acid profile, cholesterol, HDL concentration. Plasma hormones of reproductive importance like Insulin, IGF-1 and post estrous progesterone were increased with PUFA supplementation and not by palm oil supplementation. Additionally, follicular and corpus luteum size were also increased in ω-3 rich oil fed sahiwal heifers.

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