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Effect of dietary supplementation of crushed soybean and flaxseed on ovarian response in postpartum crossbred dairy cows

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Abstract

The effect of dietary supplementation of crushed soybean and flaxseed on ovarian function in postpartum crossbred dairy cows was studied. Total 20 postpartum normally calved cows from 2nd to 4th lactation were randomly divided into two equal groups. In addition to routine feed, cows from group T₁ were fed with dietary supplementation of crushed soybean @ 10% of DM requirement from the day of calving to day 50 postpartum and thereafter continuation with crushed Flaxseed @ 15% of DM requirement of animal from day 51 to day 85 postpartum respectively, whereas cows from Group T₂ was considered as control and supplemented with routine feed. All the animals from both groups were synchronized on day 60 postpartum with Ovsynch protocol. The Follicular dynamic study with the help of USG was undertaken at every 24 hrs interval before the first injection of GnRH of Ovsynch protocol and during Ovsynch protocol (Day 0 to Day 9) of synchronization. It was observed that mean number of class II follicles and total number of follicles were significantly increased in T₁ group as compared to T₂ group, whereas the mean number of class I and class III follicles did not differ significantly in T₁ and T₂ groups on day 0. During the synchronization period the mean number of class I, class II, total numbers of follicles and preovulatory follicles size were significantly larger in T₁ as compared to T₂ groups. The present research findings concluded that the dietary supplementation of crushed soybean and flaxseed showed significant increase in class I, class II, total number of follicles and size of preovulatory follicle in crossbred cows as compare to control group.

Keywords: Follicular dynamics, flaxseed, soybean, postpartum cows, ovsynch protocol

1. Introduction

Reproductive inefficiency is a major economic problem in dairy production, particularly during the phase of negative energy balance that occurs in early lactation. One way of improving energy status and thereby reproductive performance is to increase the energy density of the diet with fat supplementation and the reproductive performance is enhanced by dietary fat independent of energy status (Staples *et al.*, 1998) [16]. It is known that, cows fed supplemental fat may experience improved energy balance and begin to cycle sooner because of enhanced follicular growth and development (Grummer and Carroll, 1991). Dirandeh *et al.*, (2013) [3] stated that, feeding a source of omega 6 fatty acid can be a strategy to improve uterine health after calving, although the source of omega 3 fatty acids such as Linseed should be fed after uterine involution to decrease PGF_{2α} secretion. The main source of omega-6 fatty acid is dietary linoleic acid (C18:2n-6), which inter alia is the precursor of the dienic (2-series) prostaglandins, such as PGF_{2α} (Abayasekara and Wathes, 1999) [14]. Beside, excess linoleic acid can be converted to a shunt metabolite, eicosadienoic acid (C20:2), rather than to arachidonic acid (Kaduce *et al.*, 1982). Flaxseed and soybean is an excellent source of fat and polyunsaturated fatty acid (PUFA), but it is not commonly fed to cattle. PUFAs particularly the linolenic acid (Omega 3) and the linoleic acid (Omega 6) have gained attention owing to their classification as essential fatty acids. In cattle, dietary supplementation with various long chain PUFAs (both n-3 and n-6) induced changes in several aspects of folliculogenesis including both an increase in total follicular number and the size of the dominant or pre-ovulatory follicle (Ambrose *et al.*, 2006) [1]. Higher fertility was reported in cows ovulating larger follicles even without an increase in progesterone concentration in the subsequent luteal phase (Peter and Pursley, 2003) [11]. The 20% increase in the first service conception rate in soybean fed animals was observed by Howlet *et al.*, (2003).

Some previous studies reported an increase in the number of medium-sized follicles after administration of PUFAs in the form of crushed soybean, soybean oil or rice bran (Dirandeh *et al.*, 2013, Lammoglia *et al.*, 1996 and Thomas *et al.*, 1997) [3, 10, 18]. Dietary fat may enhance follicular development via metabolic hormones that act either at the ovarian level or on the central nervous system to stimulate GnRH secretion; thereby increased basal LH concentrations (Thomas and Williams, 1996) [17]. As there is scanty literature available on the effect of dietary supplementation of crushed soybean and flaxseed on ovarian function, the present study was conducted with the aim to investigate the influence of dietary crushed soybean and flaxseed on ovarian function in postpartum crossbred cows.

2. Materials and Methods

2.1 Selection and treatment of animals

For the present study normally calved postpartum, pluriparus crossbred dairy cows having normal reproductive cycle were selected from the Instructional Livestock Farm Complex of Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola. The cows were subjected to gynaecological examination using transrectal ultrasonography before inclusion in the study. The cows diagnosed with any apparent pathological condition of the reproductive tract were not included.

A total of twenty postpartum cows were selected and divided into two groups (n=10) as follows. In the present study; experiment was conducted to determine the effect of roasted crushed soyabean and flaxseed on ovarian activity. The selected 20 cows were equally divided in to two groups i.e. n=10 (Treatment group T1 and control group T2). The cows were fed daily as per the routine practices along with the mineral mixture to meet the maintenance and production requirements. The routine feed comprised of 2/3rd roughages (2/3rd Kutti + 1/3rd Grass) and 1/3rd concentrates. The dry matter consumption was estimated @ 2.5 kg/100 kg body weight. The animals were provided clean drinking water for 24 hours. The roasted crushed soybean was supplemented @10% of DM (300 g/100 kg of body weight/animal/day; as per Dirandeh *et al.*, 2013 [3] as a supplement over and above the feed which was routinely fed, on a dry matter basis from

the day 0 Postpartum (PP) today 50 and thereafter, supplementation of crushed flaxseed @ 15% DM (300 g/100 kg of body weight/animal/day as per Deshmukh *et al.*, (2017) [2] was started from day 51 PP and was continued up to 85 days postpartum. Whereas Group II control (T2) animals were fed routinely with regular diet/feed.

2.2 Estrus synchronization & follicular dynamics

All the cows from two treatment groups were subjected to an Ovsynch synchronization protocol on day 60 postpartum treated with Inj. Buserelin acetate 10µg I/m on day 0, Inj. Cloprostenol sodium 500 µg on day 7 and Inj. Buserelin acetate 10µg I/m on day 9, respectively. A follicular study was carried out before onset (day 0) and with 24 hrs intervals during Ovsynch protocol of estrus synchronization (day 0-9). Ultrasonography was carried out transrectally with a linear-array 7.5 MHZ probe. The size and number of follicles >3 mm were recorded on detail follicular maps. Follicles were grouped into three classes for analysis: class 1-small follicles (3.0 to 4.9 mm), class 2-medium follicles (5.0 to 9.9 mm) and class 3-large follicles (>10 mm). The growth rate, atresia rate, day of emergence of the new follicle, day of deviation of follicle, diameter of deviated follicles, at the time of deviation and diameter of preovulatory follicles were studied. Timed artificial insemination (TAI) was carried out at 16-24 hrs after the second GnRH injection. The approval of "Institutional Ethics Committee for Veterinary Clinical Research (IEC-VCR) was obtained vide Resolution no. 02/07 of 2019 The data collected on various parameters were analysed by Student T test using WASP (Web Agri. Stat Package) developed by ICAR.

3. Result and Discussion

3.1 Ovarian response in different groups

3.2. Ovarian response on day 0 before initiation of Ovsynch protocol.

The mean number of Class II follicles and a total number of follicles were significantly more in T₁ group on day 0 i.e. before initiation of Ovsynch protocol as compared to the T₂ group, whereas the mean number of Class I and Class III were at par in T₁ and T₂ groups (Table 1).

Table 1: The total number of different class of follicles before onset of Ovsynch protocol for synchronization (on day 0) in different groups of postpartum crossbred dairy cows

Class of Follicle	Total number different class of follicles		'T' Statistics
	T ₁	T ₂	
Class I (Small)	1.7 ± 0.21	1.3 ± 0.15	NS (1.524)
Class II (Medium)	3.5 ± 0.21 ^a	2.0 ± 0.21 ^b	4.881
Class III (Large)	0.5 ± 0.22	0.5 ± 0.16	0.000
Total number of follicles	5.7 ± 0.15 ^a	3.8 ± 0.2 ^b	7.550

T₁ crushed Soybean & flaxseed, T₂ control group. NS = non-significant.

The mean bearing different superscripts in a row, differs significantly.

This significant increase in class II and total number of follicles in supplemented group might be due to dietary fat that may have enhanced follicular development via metabolic hormones that act on the central nervous system to stimulate GnRH secretion and thereby increased basal LH concentrations. Another way in which dietary fat may affect follicular development is through metabolic hormones acting at the ovarian level. Thomas and Williams, (1996) [17] found that follicular development, along with plasma insulin and follicular IGF-2 concentrations, was enhanced by soybean oil.

Supplementation of soybean was effective in increasing the number of medium sized follicles compared to a saturated fat or a highly unsaturated fat (fish oil) supplement and this was associated with higher serum insulin and increased granulosa cell proliferation (Poretsky and Kalin, 1987) [13]. In the present study, the significant increase in class II and total number of follicle on day 0 in supplemented group is in accordance with Deshmukh *et al.* (2017) [2] who reported significant increase in class II and total number of follicles after supplementation of soybean oil and flaxseed in post partum cows. The mean total number of class I follicles showed non significant difference between the supplemented and non supplemented groups. On

the contrary Deshmukh *et al.* (2017) ^[2] reported a significant increase in class I follicles in the supplemented group.

3.3. Ovarian response during synchronization period

The mean number of Class I, class II and total number of

follicles during synchronization period (0-9 day) in group T₁ is significantly higher as compared to the control group i.e. T₂. Whereas the mean total number of Class III follicles were at par T₁ and T₂ groups (Table 2)

Table 2: The total number of different classes of follicles on day 0-9 in different groups of postpartum crossbred dairy cows during Ovsynch protocol

Class of Follicle	Total number of different class of follicles		'T' Statistics
	T ₁	T ₃	
Class I (Small)	3.35 ± 0.08 ^a	2.27 ± 0.07 ^b	9.571
Class II (Medium)	2.6 ± 0.08 ^a	1.33 ± 0.07 ^b	9.932
Class III (Large)	1.02 ± 0.03	1.00 ± 0.02	NS (0.480)
Total number of follicles	6.82 ± 0.15 ^a	4.60 ± 0.11 ^b	11.601

T₁ crushed Soyabean and flaxseed, T₂ control group.

The mean bearing different superscripts in a row, differs significantly.

These findings regarding the significant increase in Class I, class II (medium) follicles and total number of follicles were in close accordance with Deshmukh *et al.* (2017) ^[2] with feeding soybean oil and crushed flaxseed, Ghasemzadeh *et al.* (2011) ^[5] with feeding fish oil and soybean oil. Ponter *et al.* (2006) ^[12] recorded increase in small size follicles whereas medium size follicles was in accordance with others (Thomas *et al.* 1997; Robinson *et al.* 2002; Kassa *et al.* 2002 and Gulliver *et al.* 2012) ^[18, 14, 9, 7] who reported increase in medium size follicles with feeding different source of linolenic and linoleic acid. Ulfina *et al.* (2015) ^[20] observed

the total number of follicles tended to be ($p=0.08$) higher for the flaxseed group as compared to the other groups.

3.4 Follicular dynamic during estrus synchronization in different groups

The mean growth rate and day of emergence of follicle in T₁ group were significantly higher as compared to T₂ group during synchronization period. The rate of atresia of follicles, the mean day of deviation, mean diameter of deviated follicles and mean diameter of the subordinate follicle at the time of deviation did not differ significantly between the groups. The mean preovulatory follicle diameter was significantly larger in T₁ group as compared to T₂ group (Table 3).

Table 3: The mean follicular dynamics status in both groups of crossbred dairy cows during synchronization period

Sr. No.	Parameters	T ₁	T ₃	'T' Statistics
1	Mean growth rate (mm)	1.27 ± 0.08 ^a	0.83 ± 0.05 ^b	4.428
2	Mean atresia rate (mm)	0.65 ± 0.01	0.69 ± 0.02	NS (-1.459)
3	Mean day of emergence of new follicle (days)	2.0 ± 0.10 ^a	2.55 ± 0.13 ^b	-3.161
4	Mean day of deviation (days)	3.0 ± 0.25	3.3 ± 0.33	NS (-0.709)
5	Mean diameter of deviated follicle (mm)	8.68 ± 0.06	8.67 ± 0.03	NS (0.140)
6	Mean diameter of largest subordinate follicle at the time of deviation (mm)	7.32 ± 0.05	7.36 ± 0.04	NS (-0.548)
7	Mean diameter of pre ovulatory follicle (mm)	16.54 ± 0.26 ^a	13.62 ± 0.17 ^b	9.113

T₁ crushed soyabean and flaxseed, T₂ control group. NS = non-significant.

The mean bearing different superscripts in a row, differs significantly.

The present finding regarding mean growth rate (mm/day) of ovulatory follicle, mean day of emergence of new follicle was in accordance with Deshmukh *et al.* (2017) ^[2] who reported a significant difference in growth rate and the mean day of emergence of new follicle with supplementation of soybean oil and crushed flaxseed as compared to no fat supplementation group. Similarly, the mean day of emergence of new follicle after injection of first GnRH was in close agreement with Twagiramunga *et al.* (1995) ^[19]. The atresia rate (mm/day) of reducing subordinate follicle recorded in present study were in accordance with Gaur and Purohit (2007) ^[4] and Henrique *et al.* (2000) ^[8]. On the contrary to the present finding, Deshmukh *et al.* (2017) ^[2] reported significant difference in atresia rate in soybean oil fed cows as compared to no fat fed cows which is in accordance with the present findings. The mean day of deviation of dominant follicle after GnRH injection recorded was in agreement with Deshmukh *et al.* (2017) ^[2] and Segwagwe *et al.* (2006) ^[15]. The mean diameter of deviated and subordinate follicle on the day of deviation recorded was in similarity with Deshmukh *et al.* (2017) ^[2] and Ginther *et al.* (2000) ^[6] who reported the mean

diameter of deviated and subordinate follicle on the day of deviation was 8.65, 7.32 and 8.5, 7.7 mm, respectively. The mean diameter of preovulatory follicle recorded in the present study was in close agreement with Deshmukh *et al.* (2017) ^[2] who reported significant increase in preovulatory follicle size of cows supplemented with soybean oil and crushed flaxseed group as compared to no fat fed cows. Similarly, Ghasemzadeh *et al.* (2011) ^[5] recorded significant increase in preovulatory follicle size in fish oil and soybean oil groups as compared to control group of cows. Similarly, Ulfina *et al.* (2015) ^[20] observed that, the size of dominant follicle was significantly higher ($p < 0.05$) for flaxseed group as compared to control group.

4. Conclusion

From the study, it was concluded that dietary supplementation of roasted crushed soybean and flaxseed helps in increasing Class II, the total number of follicles and size of the preovulatory follicle in postpartum cows.

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6. Conflict of Interest: All authors declare no conflict of interest.

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