Full Length Article

NUTRITIONAL INTERVENTION WITH FLAXSEED FOR UTERINE HEALING AND FERTILITY ENHANCEMENT IN SUBCLINICAL ENDOMETRITIS AFFECTED CROSSBRED DAIRY COWS

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ABSTRACT

Subclinical endometritis (SCE) is characterized by increased polymorphonuclear neutrophils (PMNs) in the endometrium without clinical signs of uterine infection. This condition adversely affects fertility and herd productivity. The present study aimed to assess the efficacy of dietary flaxseed supplementation in improving uterine health and reproductive performance in lactating dairy cows diagnosed with SCE. Twelve SCE-positive cows, confirmed by the White Side Test (WST) and endometrial cytology (>5% PMNs), were randomly assigned to two groups: Group I (control; n=6) received a standard ration, and Group II (treatment; n=6) supplemented with flaxseed concentrate for three months. Post-treatment evaluation after second subsequent estrus revealed a significant improvement of the uterine status in the flaxseed-supplemented group. WST positivity declined from 100% to 16.67% in Group II, as compared to 66.67% in the control group. PMN percentage significantly decreased from 15.74 ± 0.99% to 3.67 ± 0.55% in the flaxseed group, whereas a lesser decline (13.67 ± 0.67% to 5.33 ± 0.39%) was observed in controls. Conception rate was markedly higher in the flaxseed group (83.33%) compared to the control group (33.33%). The results demonstrated that the flaxseed supplementation effectively reduced uterine inflammation and enhances fertility in SCE-affected cows, offering a practical nutritional strategy for dairy herd reproductive management.

Keywords: Subclinical endometritis; flaxseed; PMN cells; conception rate

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INTRODUCTION

Subclinical endometritis is characterized by a higher percentage of polymorphonuclear neutrophils (PMN) cells in the endometrium without any clinical symptoms (Kasimanickam *et al*., 2004). The endometrium becomes infiltrated with neutrophils and granulocytes, detectable only by cytological examination. It may occur after completion of uterine involution, and affected cows show no cervical discharge due to minimal exudate accumulation (Gilbert *et al*., 2005; Sheldon *et al*., 2006).

Subclinical endometritis has a multifactorial etiology, with bacterial infection being a major contributor (Földi *et al*., 2006; Gautam *et al*., 2009). Although more than 70% of cows clear postpartum uterine infection through innate immunity, 17–37% develop clinical endometritis, while 14–53% develop subclinical endometritis (Cheong *et al*., 2011; Madoz *et al*., 2014). Thus, uterine infections contribute significantly to reproductive and economic losses in dairy herds.

Owing to limitations associated with antibiotic therapy, greater attention has shifted to immunomodulatory strategies for managing endometritis and repeat breeding. Immunomodulators enhance uterine defense mechanism by stimulating interleukin production (Sica *et al*., 1990), thereby promoting recruitment and activation of polymorphonuclear leukocytes, macrophages, and monocytes essential for pathogen elimination (Dhaliwal and Sidhu, 2002).

Uterine infection triggers increased neutrophil migration into the uterine lumen and endometrium (Zerbe *et al*., 2003), while endogenous antimicrobial peptides and acute-phase proteins support innate immune defense (Sheldon *et al*., 2001). However, persistent infections may disrupt endocrine balance, impair embryo survival, and alter ovarian function, resulting in anovulation or extended luteal phases.

Flaxseed favourably modulates immune responses and it contains α-linolenic acid component which modifies membrane phospholipids, inhibits arachidonic acid synthesis, reduces pro-inflammatory eicosanoid production, and suppresses lymphocyte proliferation and cytokine synthesis (Leaf and Weber, 1988). Rich in omega-3 fatty acids, flaxseed has been associated with enhanced uterine recovery and improved fertility in dairy cattle (Ambrose *et al*., 2006; Bilby *et al*., 2006). Therefore, the study was hypothesized that dietary flaxseed inclusion would reduce uterine inflammation, enhance immune cell activity, and accelerate recovery from subclinical endometritis. The present study aimed to evaluate endometrial cytology and uterine response before and after flaxseed supplementation to assess its therapeutic potential and role in fertility improvement.

MATERIALS AND METHODS

A total of twelve lactating crossbred dairy cows, 2-5 years old with a body condition score in the range of 3-4, fed with roughage and concentrates brought to the

Large Animal Gynaecology ward of Madras Veterinary College teaching hospital were selected for the study based on clinical examination and laboratory screening for subclinical endometritis using white side test and endometrial cytological examination. All these cows showed no external signs of uterine infection but exhibited altered uterine cytology. In all the cows, subclinical endometritis was diagnosed using white side test (Ferdousy and Kadokawa, 2022) and endometrial cytology by cytobrush technique (Kasimanickam *et al.*, 2004).

The animals were randomly divided into two equal groups (n=6 per group) and the diets were given as mentioned in table 1. Farmers were instructed to present the animals at the second subsequent estrus following initiation of feeding with or without flax seed supplementation. At this estrus, uterine health was reassessed by WST and endometrial cytology by cytobrush technique.

The PMN% and WST reactions were compared with baseline values to assess improvement in uterine environment. At least 200 cells were counted; animals with >5% polymorphonuclear cells (PMNs) were classified as subclinical endometritis cases. For WST, development of yellow to dark-brown coloration indicated elevated inflammatory cell content and positive reaction.

On Day 8 of the second subsequent estrus, estrus was induced using $PGF_2\alpha$ injection (Pragma, Intas Pharmaceuticals Ltd.) @ 500 µg I/M. Artificial insemination (AI) was performed 72–96 hours after $PGF_2\alpha$ administration, timed according to estrus expression.

Pregnancy diagnosis was carried out approximately 45–60 days post-insemination by ultrasonography and/or rectal palpation.

RESULTS AND DISCUSSION

White Side Test (WST)

The results of WST in both the groups are shown in Table 2. The White Side Test (WST) outcomes elucidated a pronounced amelioration in uterine health subsequent to flaxseed supplementation. Prior to initiation of the dietary intervention, all twelve cows (100%) exhibited WST positivity, confirming the presence of subclinical endometrial inflammation. Following the treatment period, four of six cows (66.67%) in the control cohort persisted as WST-positive, signifying continued intrauterine inflammatory status in the absence of nutritional modulation. Conversely, five of six cows (83.33%) within the flaxseed-supplemented cohort transitioned to WST-negative status, with only a single cow retaining positivity, thereby demonstrating a substantial attenuation of uterine inflammatory exudates. These observations underscore the salutary influence of flaxseed-enriched diets on uterine milieu restoration and align with earlier scholarly assertions delineating the deleterious consequences of persistent uterine inflammation on reproductive efficiency in dairy bovines (Kasimanickam *et al*., 2004; Gilbert *et al.*, 2005; Sheldon *et al*., 2006).

Endometrial Cytology (PMN%)

The PMN cell count before and after treatment in both the groups is presented in Table 3. Endometrial cytology revealed elevated polymorphonuclear neutrophil (PMN) percentages in both groups prior to treatment, confirming subclinical endometritis, with no significant difference between groups ($P > 0.05$). In the control group, PMN values significantly decreased from 13.67 ± 0.67% to 5.33 ± 0.39% after treatment ($t = 8.626$; $P < 0.05$). Similarly, the flaxseed-supplemented group showed a significant reduction in PMN counts from 15.74 ± 0.99% to 3.67 ± 0.55% following treatment ($t = 23.238$; $P < 0.05$). Post-treatment PMN values were significantly lower in the flaxseed group compared to the control group ($P < 0.05$). These findings are in accordance with earlier studies reporting that reduction in endometrial inflammation is associated with improved uterine cytology and subsequent reproductive performance in dairy cows (Gilbert *et al*., 2005; Cheong *et al*., 2011; Madoz *et al*., 2014).

Conception Rate

The results are summarized in Table 4. Estrus was induced on the eighth day of the second subsequent estrous cycle, followed by artificial insemination 72–96 hours after $PGF_2\alpha$ administration. In the control group, only 2 out of 6 cows (33.33%) conceived, indicating limited improvement in reproductive efficiency without dietary intervention. Conversely, the flaxseed-supplemented group exhibited a markedly superior conception rate, with 5 out of 6 cows (83.33%) becoming pregnant. This substantial disparity underscores the pivotal role of flaxseed in ameliorating uterine conditions and fostering an intrauterine milieu conducive to fertilization and embryo survival. These findings are in agreement with previous literature emphasizing that mitigation of uterine inflammation significantly enhances conception outcomes in dairy cattle (Gilbert *et al*., 2005; Madoz *et al*., 2014).

The enhanced reproductive performance in the flaxseed-supplemented cohort may be attributed to the pronounced anti-inflammatory and reproductive-modulatory effects of omega-3 fatty acids, particularly α-linolenic acid. Earlier studies have similarly demonstrated that omega-3 supplementation attenuates inflammatory mediators, optimizes eicosanoid profiles, and improves conception and embryo retention in dairy cows (Wathes *et al.*, 2007; Santos *et al*., 2008). Omega-3 fatty acids regulate inflammatory pathways, modulate prostaglandin synthesis, and support embryonic development. The present findings are further corroborated by reports of increased fertility and reduced embryonic losses following flaxseed supplementation (Ambrose *et al*., 2006; Bilby *et al.*, 2006).

CONCLUSION

Flaxseed supplementation resulted in a marked reduction in endometrial PMN percentage and White Side Test reactivity, signifying effective resolution of subclinical uterine inflammation.

Reproductive performance outcomes further substantiated the therapeutic efficacy

of flaxseed, as evidenced by the significantly higher conception rate in the supplemented group (83.33%) compared to the control group (33.33%). The findings of the present investigation clearly establish that dietary supplementation of flaxseed serves as an effective strategy for ameliorating uterine health and enhancing fertility in dairy cows affected with subclinical endometritis.

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Table 1. Experimental design

GROUP (n=12)	FEED
Group I (n=6)	Standard dairy ration for 3 months
Group II (n=6)	Standard dairy ration + 2 kg flaxseed-based concentrate for 3 months

Table 2. Number of White Side Test Positive samples before and after treatment

GROUP (n=6)	WHITE SIDE TEST POSITIVE (%)	
	BEFORE TREATMENT	AFTER TREATMENT
Group I (Control)	100	66.67
Group II	100	16.67

Table 3. Polymorphonuclear neutrophil (PMN) cell count before and after treatment

GROUP (n=6)	Polymorphonuclear neutrophil (PMN) Cell count (%)		
	BEFORE TREATMENT	AFTER TREATMENT	t value
Group I (Control)	13.67 ± 0.60B	5.33 ± 0.39bA	8.626**
Group II	15.74 ± 0.99B	3.67 ± 0.55aA	23.238**

n=6; Values are depicted as Mean ± SE. Means bearing different superscript in each column depicted by a, b, c and in each row by A, B, C differ significantly
NS Non-significant (P > 0.05) *Significant at P < 0.05 level **Significant at P<0.01 level

Table 4. Conception rate after treatment

GROUP (n=6)	CONCEPTION RATE (%)
Group I	33.33
Group II	83.33

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