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Flaxseed supplementation in buffaloes attenuates plasma $PGF_{2\alpha}$ concentrations during last quarter of estrus cycle

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ABSTRACT

The present study was designed to test the effect of flaxseed supplementation on luteal phase plasma $PGF_{2\alpha}$. The study was conducted on 6 lactating Murrah buffaloes used as self-control and the experiment was divided into 2 periods; pre-supplementation and supplementation period. During pre-supplementation period, all the buffaloes were synchronized using Ovsynch+CIDR protocol. On day 15 of synchronized estrous cycle, animals were subjected to oxytocin challenge test and blood samples were collected at 15 min interval starting 1 h before till 4 h after oxytocin challenge. Thereafter, same buffaloes were supplemented with flaxseed (300g/100 kg bwt/day) for 60 days. After 35 days of supplementation, buffaloes were again synchronized using same protocol and were subjected to oxytocin challenge test and similar blood sampling protocol on day 15 of synchronized cycle as during pre-supplementation period. Mean plasma $PGF_{2\alpha}$-metabolite was lower during supplementation period compared to pre-supplementation period. However, oxytocin failed to have any impact on plasma PGFM concentrations both during pre-supplementation and flaxseed supplementation period.

Key words: Buffalo, PGFM, Flaxseed, Oxytocin challenge

Prostaglandins are well known for their effects on corpus luteum (CL) function (Robinson *et al*. 2002). Arachidonic acid (ARA), a precursor of prostaglandins, in the presence of rate limiting enzyme cyclooxygenase-2 (COX-2) is oxidized to prostaglandin H_2 (PGH_2), which is the precursor of all other prostaglandins, the main luteolytic agents in dairy cattle.

Prolonged dietary supplementation of PUFAs to dairy cattle reduce endometrial synthesis and secretion of PGF2α (Mattos *et al*. 2003, 2004). This decrease in endometrial synthesis of $PGF2_{\alpha}$ is through an omega-3 fatty acid viz. α-linolenic acid (Ambrose *et al*. 2006). Alpha-linolenic acid (C18: 3n-3) is a precursor to eicosapentaenoic acid (EPA; C20: 5, n-3) and docosahexaenoic acid (DHA; C22: 6, n-3). Both EPA and DHA interfere with the conversion of arachidonic acid (precursor of PGF2α) to PGF2α (Binelli *et al*. 2001). Flaxseed (*alsi* or linseed) is a major source of α-linolenic acid (Petit 2003). Various studies in dairy cattle have revealed that dietary supplementation (for 40 to 60 days) of whole flaxseed or fish products can lead to suppression of plasma prostaglandin $F2_{\alpha}$ metabolites (PGFM, a marker of circulating $PGF2_{\alpha}$), improve luteal and metabolic profile, and ultimately lead to an increase in conception rate (Wamsley *et al*. 2005, Heravi *et al*. 2007). Accordingly the objective of this study was to assess the effect of flaxseed supplementation on endometrial secretion of $PGF_2\alpha$ in lactating buffaloes.

MATERIALS AND METHODS

Apparently healthy, lactating Murrah buffaloes (6) having average body weight between 300 to 400 kg were selected for the study. Buffaloes were daily fed 40 kg green fodder and 2 kg concentrate mixture and were provided *ad lib.* drinking water throughout the day. All the buffaloes were housed under semi-loose housing system. These animals were used as self-control group and the experiment was divided into 2 periods: pre-supplementation and flaxseed-supplementation period. During pre-supplementation period, animals were reared on routine diet and during

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supplementation period they were supplemented with crushed flaxseed (@ 300g/100kg body weight/day) for 60 days in addition to normal routine diet. Buffaloes during both periods were synchronized (about 35 days after the start of flaxseed supplementation during supplemented period) using Ovsynch plus CIDR (controlled internal drug release; 1.38 g progesterone) protocol. In this protocol, on day 0, a GnRH analogue (inj buserelin acetate, 0.02 mg) was administered (i.m.) and all the buffaloes were inserted a CIDR device intravaginally. On day 7, CIDR was removed and each buffalo was administered (i.m.) a $PGF_{2\alpha}$ analogue (inj cloprostenol sodium, 0.52 mg). On day 9, all the buffaloes were again administered (i.m.) GnRH analogue (inj buserelin acetate, 0.02 mg). In this protocol, buffaloes were expected to exhibit estrus on day 10. On day 15 after the onset of synchronized estrus, during both periods, jugular vein of all the buffaloes was catheterized. Release of $PGF_{2\alpha}$ was stimulated by oxytocin (100 IU) administration via intravenous route (Fig. 1). Blood sample (5 ml) collection was carried out at 15 min interval starting 1 h before till 4 h after oxytocin challenge by fixing a polystyrene catheter in jugular vein of each buffalo for estimating plasma PGFM concentrations. Samples were immediately stored in ice and centrifuged at 3,000 rpm for 15 min for separation of plasma. Two aliquots of plasma were stored at –20°C until assayed.

Plasma PGFM (a marker of $PGF_{2\alpha}$ metabolite) was estimated using a commercially available enzyme immune assay kit.

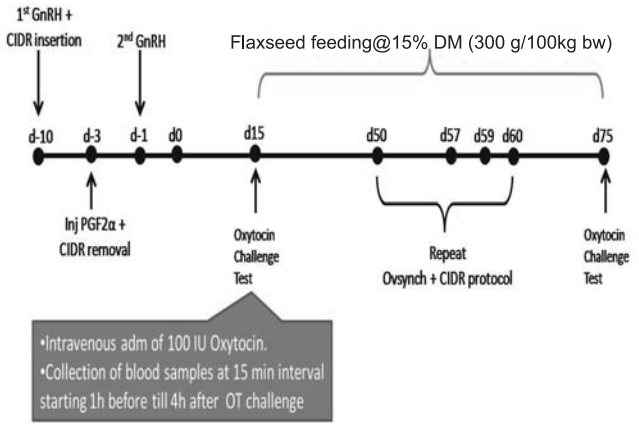

Fig. 1. Schematic diagram for treatment schedule of buffaloes (n=6) during pre-supplementation and flaxseed supplementation period. Ábbreviation; bw: body weight, CIDR: controlled internal drug release, d: day; GnRH: gonadotropic releasing hormone, DM: dry matter, OT: oxytocin, $PGF_{2\alpha}$: prostaglandin$F_{2\alpha}$.

RESULTS AND DISCUSSION

During pre-supplementation period, plasma PGFM concentrations recorded at 15 min interval before oxytocin challenge ranged from 778.3±224.0 to 913.0±332.2 pg/ml (Table 1). In comparison, plasma PGFM concentrations during supplementation period were significantly (P<0.05) lower (293.0±78.5 to 435.0±63.8 pg/ml, Table 1). Similarly, average plasma PGFM concentrations released in 1h before oxytocin challenge was significantly (P<0.05) lower in the supplementation period in comparison to pre-supplementation period (389.0±74.2 versus 952.1±71.0 pg/ml, respectively; Fig. 2). The observed trend of less (P≤0.05) plasma PGFM concentrations in supplementation period compared to pre-supplementation period also continued during the post-oxytocin challenge period (Table 1; Fig. 2).

Similar to present observations, a decrease in PGFM concentrations in uterine flushings of non-inseminated dairy cattle was also observed subsequent to whole flaxseed supplementation (9.1% DM) for 4 weeks starting at 2 weeks postpartum (Petit *et al.* 2007). Álpha-linolenic acid (ÁLA) present in flaxseed plays an important role in the suppression of $PGF_2\alpha$ synthesis by inhibiting the endometrial expression of COX-2, a rate limiting enzyme in the synthesis of $PGF_2\alpha$ (Palin *et al.* 2005). Moreover, ALA inhibits $PGF_2\alpha$ release through decreased availability of arachidonic acid as well as through increased competition of ALA with arachidonic acid for binding to COX-2 (Mattos *et al.* 2002).

In present study, buffaloes were subjected to oxytocin

Table 1. Plasma 13, 14-dihydro-15-keto $PGF_{2\alpha}$ (PGFM) concentrations (pg/ml; mean±SE), at 15 min interval, starting at 1 h before till 4 h after oxytocin challenge on day 15 of synchronized estrous cycle during pre-supplementation and supplementation period in buffaloes (n=6). Buffaloes used in pre-supplementation and supplementation period were same

	Time (min)	Plasma PGFM (pg/ml)	
		Pre-supplementation period	Supplementation period
Pre- oxytocin challenge period	–60	913.0±332.2	435.0±63.8
	–45	910.8±284.5	339.2±50.8
	–30	908.3±316.3	313.0±54.4
	–15	778.3±224.0	293.0±78.5
Post- oxytocin challenge period	15	863.3±285.1	325.8±52.9
	30	844.7±277.1	326.7±58.1
	45	818.3±226.0	431.7±79.0
	60	951.7±306.5	297.5±69.7
	75	850.8±271.7	309.2±74.1
	90	1159.2±447.6	283.3±60.3
	105	919.2±331.3	297.5±66.3
	120	870.8±310.5	302.5±60.4
	135	965.8±294.1	348.3±57.1
	150	805.0±254.9	325.0±68.2
	165	1061.7±311.2	306.7±80.4
	180	980.0±348.8	289.0±77.4
	195	952.5±309.6	301.7±77. 8
	210	902.7±305.0	304.2±72.8
	225	954.2±310.7	313.3±65.1
	240	975.8±304.0	341.7±84.4

*P≤0.05; from corresponding values in a row.

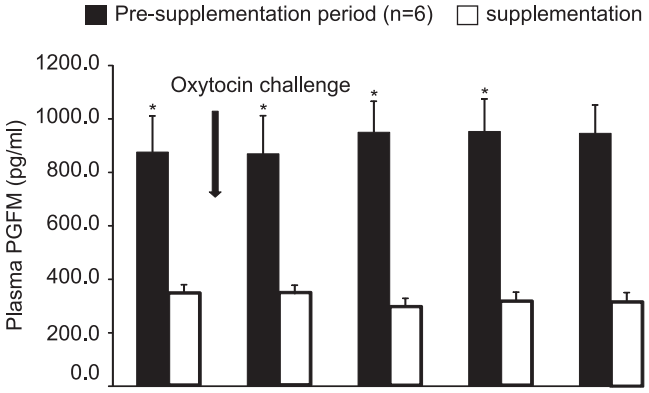

Fig. 2. Plasma 13, 14-dihydro-15-keto $PGF_{2\alpha}$ (PGFM) concentrations (pg/ml; mean±SE) at 1h interval, before and after oxytocin challenge on day 15 of synchronized estrous cycle during pre-supplementation and supplementation period in buffaloes (n=6). *P<0.05; from corresponding values of supplemented period.

challenge, on day 15 of synchronized estrus cycle both during pre-supplementation and supplementation period, however, there was no alteration (P<0.05) in plasma PGFM concentrations during post-oxytocin challenge period (Table 1; Fig. 2). Similarly, postpartum dairy buffaloes reared without any fat supplementation or supplemented with 250g fish meal for 30 days failed to exhibit an increase in plasma PGFM concentrations subsequent to oxytocin challenge on day 15 of synchronized estrous cycle (Malik *et al.* 2011). Lactating cattle supplemented with protected flaxseed also failed to exhibit any impact on plasma PGFM concentration following administration of oxytocin (50 IU) on day 15 of estrous cycle (Robinson *et al.* 2002).

The failure of response to oxytocin challenge could be due to the day of estrous cycle (day 15) selected in present study for oxytocin challenge. Robinson *et al.* (2002) recorded an increase in plasma PGFM when oxytocin was administered on day 17 of the estrous cycle and no increase in plasma PGFM was observed following oxytocin challenge on day 15. Others advocated use of estradiol injection prior to oxytocin challenge for better response to challenge test in terms of plasma PGFM concentrations (Petit *et al*. 2004, Petit and Twagirumunga 2006). Estradiol is known to up-regulate oxytocin receptors, thereby, increasing concentrations of plasma PGFM (Goff 2004). In contrast, a report has suggested that the use of estradiol in conjugation with oxytocin challenge has no impact on plasma PGFM response (Wamsley *et al.* 2005).

Luteal phase plasma PGFM concentrations recorded in buffaloes of present study (Table 1; Fig. 2) were in agreement with previous data in buffaloes in which luteal phase plasma PGFM concentrations were around 300–950 pg/ml (Dobson 2002). In comparison to present findings, lower plasma PGFM concentrations were recorded on day 15 of estrous cycle in buffaloes (Batra and Pandey 1983). In another study on postpartum buffaloes, luteal phase plasma PGFM was around 180–300 pg/ml (Malik *et al.* 2011). Furthermore, variations in luteal phase plasma PGFM also exist in various studies carried out on dairy cattle. Plasma PGFM in dairy cattle supplemented with whole or formaldehyde-treated flaxseed ranged from 20–80 pg/ml around oxytocin challenge test on day 15 of synchronized estrous cycle (Petit *et al.* 2004, 2002). In contrast, in another study on dairy cattle, plasma PGFM was in the range of 4–50 ng/ml on day 15 of estrous cycle (Heravi *et al.* 2007). The observed variations recorded in luteal phase plasma PGFM in various cattle and buffalo studies could be due to different methods of hormone assay (direct/indirect radio immuno assay or enzyme immuno assay) and different source of PGFM antibody (Petit *et al.* 2002, 2004, Heravi *et al.* 2007 and Malik *et al.* 2011).

In conclusion crushed flaxseed decreases endometrial secretion of $PGF_{2\alpha}$ in response to oxytocin challenge on day 15 post-estrus but oxytocin failed to have any impact on plasma PGFM concentrations

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