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EFFECT OF DIETARY FAT SUPPLEMENTATION ON THE REPRODUCTION RESPONSE TO PROSTAGLANDIN (PGF_2A) INJECTION IN POST PARTUM BUFFALO-COWS.

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ABSTRACT

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40 multiparous buffalo's cows in the postpartum period were used in this study. The animals aged 6-8 years and have a history of persistent corpus lutetium (C.L.). Two groups of animals were divided, the first one was used as a control (untreated), the second one was fed a basal diet with cotton seed oil 2% from parturition till the end of the experiment. All buffaloes showed mature C.L. were injected with $PGF_2\alpha$. The buffaloes (control and treated) showed estrus were artificially inseminated 72 hours post injection (timed A.I) and examined rectaly to asses pregnancy after 60 days post insemination. Blood samples were collected from all animals before injection of $PGF_2\alpha$ (60 days PP) and one month after injections (90 days PP) for analysis of serum glucose, triglycerides, fatty acids and cholesterol. Serum zinc and copper levels and total antioxidants were determined. Our results revealed that serum glucose concentration was not significantly affected by fat supplementation. While triglycerides and cholesterol were significantly increased with cotton seed oil supplementation compared with control group at 90 days PP. Also the results revealed that serum concentration of palmitic, stearic. oleic, linolic and linolenic fatty acids were significantly increased with cotton seed oil supplementation group, and was more markedly increased when it was added to diet for 3months. The results also illustrated a non-significant effect on serum concentration of copper and zinc by fat supplementation compared to control group Where total antioxidants was significantly increased in the supplemented group. On the other hand the overall mean of conception rates in buffaloes supplemented with cotton seed oil were significantly increased (82.3%) than non-supplemented ones.

Key Words: *Dietary fat, PGF_2A, buffalo-cows.*

INTRODUCTION

Estrus synchronization decreased the inter calving period and allowed artificial insemination planning according to a time and scheme, also allows to stagger the plan parturitions beginning in a specific period of time (Martineau, 2003).

The hormone $PGF_2\alpha$ is important for uterine involution and ovarian function, that is able to synchronize estrus by inducing regression of the corpus luteum (Xiang – Dong, 2003).

However, the possibility of modifying the estrus cycle through hormonal treatment still has some obstacles. Fatty acid levels and antioxidants are factors affecting calving interval and first service of artificial insemination in cattle and buffaloes.

Nutritional approaches have been used to improve reproductive performance in cattle. Plasma lipid profiles, hormone concentration and follicular dynamics can be attended by including fat in live stock diets (Lucy *et al.,* 1991). Some polyunsaturated long chain fatty acids can serve as substrates for biosynthesis of the prostaglandins for mammals (Mattos *et al.,* 2000). Arachidonic acid is the main substrate for prostaglandins (Attar *et al.,* 2009 and Cammas *et al.,* 2006). Increasing dietary energy density by feeding supplemental fat may enhance metabolic efficiency. Theoretically, if feeding supplemental fat increases energy intake, severity of negative energy balance may be reduced and better health and improved reproductive efficiency may result (Curtis *et al.,* 1985).

Also the other main factor affecting reproduction is antioxidants. They are also key signal molecules modulating various reproductive functions and can be affected by type of supplemented fat (Vazquez *et al.,* 2008). Free radicals can influence the oocyte, sperm, and embryo in their micro environment (for example follicular fluid, hydrosalpingeal fluid, and

peritoneal fluid). These microenvironments have a direct bearing on the quality of oocytes, sperm oocyte interaction, implantation and early embryo development. Oxidative stress affects both implementation and early embryo development (Ashok *et al.*, 2006).

Thus our objective was to determine whether increasing the level of fat percent of the dry matter of the diet by dietary fat supplement to post partum dairy buffaloes would improve the reproductive response after synchronization using $PGF_{2\alpha}$.

MATERIALS and METHODS

Experimental animals:-
For this study 40 multiparous lactating buffalo-cows (aged 6 to 8 years) after parturition were selected and has a history of persistent C.L. Buffaloes were divided into two groups according to their fat percent of the diet, the first group left without treatment (control group) n=16 were fed the basal diet TMR. The second group (n=24) was given the basal diet plus cotton seed oil 2% fat of the dry matter. Cotton seed oil was offered from parturition till the end of experiment (60 days post insemination).

Feeding regime:-
TMR Mixture was offered as 15-17 kg dry matter per animal. It's chemical analysis was crude protein 16% and ether extract 2%. The basal diet offered to all animals and covered their requirement. TMR containing fat was prepared by adding cotton seed oil (2% of the dry matter plus the basal diet). All buffaloes showed mature C.L. were injected with 500 µg single dose of $PGF_2\alpha$ analogue (Estrumate, Schering-Plough Animal Health). The buffaloes (control and treated) that showed estrus were artificially inseminated 72 hours post injection (timed A.I). The inseminated animal were examined rectaly to asses pregnancy after 60 days post insemination (conception rate).

Sampling, measurements and analysis:
Blood samples were collected from all animals just before injection of $PGF_2\alpha$ (60 days PP.) and one month after injections (90 days PP). Serum glucose, triglyceride and cholesterol were analysed using kits Fatty acids were extracted from serum with methanol: chloroform (2:1 vol/vol) Loor *et al.* (2004), methylated and then subjected to gas chromatography. Total antioxidants were determined using specific kits. Serum zinc and copper levels were determined by using atomic absorption spectrophotometer.

Statical analysis:
The obtained data were statically analysed by student T test according to the method described by (Snedecor and Cochran, 1987).

RESULTS

Serum glucose, triglyceride and cholesterol analysis:
Our results revealed that serum glucose concentration was not significantly affected by fat supplementation .While triglycerides were significantly increased with cotton seed oil supplementation compared with control group at 90 days PP, and cholesterol at both 60 &90 days PP (table 1).

Fatty acid analysis:
Our results revealed that serum concentration of palmitic, stearic. oleic, linoleic and linolenic fatty acids were significantly increased with cotton seed oil supplementation group (table 2), and was more markedly increased when it was added to diet for 3months.

Serum copper, zinc and total antioxidants analysis:
Results illustrated non significant effect on serum concentration of copper and zinc by fat supplementation compared to control Where total antioxidants was significantly increased in the supplemented group at 90 days PP (table 3).

Conception rate:
On the other hand (table 4) declared that the overall mean of total conception rates (first and second insemination) in buffaloes supplemented with cotton seed oil were significantly increased (83.3%) than non supplemented ones (68.75%).

Table 1: Serum glucose, triglyceride and cholesterol in buffaloes supplemented with 2% cotton seed oil compared with control group (mean ± SE).

Supplementation period	60 days postpartum		90 days postpartum	
Glucose (mg/dl)	Control	supplemented	Control	supplemented
	74.5±2.7	75.2±2.5	73.2±2.6	74.2±1.9
Triglyceride (mg/dl)	38.3±1.4 ^{a}	39±1^{a}	36.56±1.1 ^{A}	41.7±1.2 ^{B}
Cholesterol ((mg/dl)	46.98±2.04^{a}	50.15±1.09 ^{b}	45.2±1.5 ^{A}	49.4±1.6^{B}

* Data with different subscript (a,b at 60 days and A,B at 90 days PP) in the same row are significantly differ at (P<0.05).

Table 2: Serum fatty acid analysis in buffaloes supplemented with 2% cotton seed oil compared with control group (mean ± SE).

Parameter (µg/ml)	60 days postpartum		90 days postpartum	
Palmitic acid	Control	supplemented	Control	supplemented
	1240.0 ±66.2 [b]	1578.0 ±60.0 [a]	1178.0 ± 70.0 [B]	1750.0 ± 63.0 [A]
Stearic acid	779.0 ±39.5 [b]	850.0 ±55.4 [a]	750.0 ± 57.3 [B]	800.0 ± 40.5 [A]
Oleic acid	613.0 ±32 [b]	890.0 ±43.0 [a]	600.0 ± 38.0 [B]	800.0 ± 40.0 [A]
Linoleic	30.0 ±5.0 [b]	50.0 ±7.8 [a]	28.0 ±1.7 [B]	70.0 ± 2.3 [A]
Linolenic acid	70.0 ±11.9 [b]	150.0 ±5.0 [a]	65.0 ±10.4 [B]	160.0 ± 28.0 [A]

* Data with different subscript (a,b at 60 days and A,B at 90 days PP) in the same row are significantly differ at (P<0.05).

Table 3: Serum copper, Zinc and total antioxidant in buffaloes supplemented with 2% cotton seed oil compared with control group (mean ± SE)

	60 days postpartum		90days postpartum	
Copper (mg/ml)	Control	supplemented	Control	supplemented
	0.85±0.07	0.79±0.03	0.92±0.02	0.84±0.1
Zinc (mg/ml)	0.62±0.01	0.58±0.3	0.66±0.01	0.60±0.02
Total antioxidant (nmol/L)	0.80±0.2a	0.77±0.1 a	0.80±0.16 [A]	0.87±0.19 [B]

* Data with different subscript (a,b at 60 days and A,B at 90 days PP) in the same row are significantly differ at (P<0.05).

Table 4: Total conception rates in control and supplemented groups.

Number of insemination	Control		Supplemented	
	No.	%	No.	%
1st insemination	8	50 b	18	75 a
2nd insemination	3	18.75 a	2	8.3 b
Total C.R.	11	68.75 b	20	83.3 a

* Data with different subscript (a, b) in the same row are significantly differ at P<0.05).

DISCUSSION

In the present study there was significant increase in serum triglycerides and cholesterol concentration in buffaloes fed cotton oil diets compared with control. This result was consistent with previous result (Grummer and Carroll, 1991) as dietary fat supplementation in cows consistently increases plasma concentrations of cholesterol. Fats which include cholesterol, triglycerol and phospholipids are major component of cellular membranes and are a source of fatty acids for the synthesis of a variety of effectors molecules such as prostaglandins. Cholesterol is another component of the cellular membrane and is the precursor for the synthesis of steroids hormones. Increased concentration of plasma progesterone in luteal phase before and after insemination have been associated with higher pregnancy rate (Buttler *et al.*, 1996).

Fats in the diet can influence reproduction positively by altering both ovarian follicle and corpus luteum function via improved energy status and increasing precursors for the synthesis of reproductive hormones such as steroids and prostaglandins (Staples *et al.*, 1998).

Feeding cotton oil for 3 months increased significantly palmitic, oleic, linoleic and linolenic fatty acids. These observation agreed with previous studies reported by Abughazaleh *et al.* (2003) and Loor *et al.* (2005) who supplemented fat to cows.

Prostaglandin F2a (PGF) appears to be the normal physiological signal whereby the uterus causes

regression of the CL at the end of the estrous cycle. During the early postpartum period, high PGF concentrations probably prolong the interval to normal CL function because hysterectomy reduces PGF concentrations and maintains normal CL function (Edqvist *et al.,* 1982; Lindell *et al.,* 1982a,b; Madej *et al.,* 1984 and Copelin *et al.,* 1987).

The significant higher concentration of serum palmitic and oleic acid as saturated fatty acids in the supplemented group compared to control group is due to hydrogenation of the unsaturated fatty acids found in cotton oil to a saturated form in the rumen or absorbed from the diet. The chemical composition of cotton seed oil and it's fatty acid profile generally consists of 18% mono saturated (oleic) and 26%saturated palmitic and stearic and 52% poly unsaturated linoliec acid. The bovine CL contains enormous quantities of arachidonic acid (Lukaszewska and Hansel, 1980) and has the biosynthetic capacity to metabolize this into a variety of products. It is the purpose to focus on two of these metabolites, PGI2 and 5-HETE (5-Hydroxyeicosatetraenoic acid), and to ascribe roles for them in the regulation of the bovine CL. Post partum anestrus commonly is referred to as postpartum interval (PPI), the interval from parturition to first estrus. Intervals to other endpoints such as to ovulation or to conception also are important, but from a practical point of view, estrus is the most logical measure of the commencement of potential fertility.

The significant higher serum linoliec and linolenic acid in the supplemented group compared to control indicated that some of the polyunsaturated fatty acids in the cotton oil escape the ruminal biohydrogenation and absorbed from the small intestine (Klusmeyer and Clark, 1991). Calculated the biohydrogenation of unsaturated C_{18} fatty acids to be approximately 70% using ruminally and duodenally cannulated lactating dairy cows.

Nutritional effects are elicited via a complex interplay among many variables such as quantity and quality of feed intake, nutrient reserves stored in the body and competition for nutrients from other physiological functions besides reproduction (Dunn and Kaltenbach, 1980; Short and Adams, 1988 and Randel, 1990).

Previous research showed that greater proportion of cows fed long chain fatty acid showed stronger signs of estrus (71.4 vs 65.5%), exhibited standing estrus, had more active ovaries (75.4 vs 69.5% as determined by rectal palpation done every 2 to 4 week) and required less exogenous $PGF_{2\alpha}$ to induce estrous (43.7 vs 55.7%) (Staples *et al.,* 1998).

Greater concentration of lioniec and linolenic acids in the oil may depress $PGF_{2\alpha}$ to a greater extent, thus allowing greater synthesis and secretion of progesterone from granulosa and luteal cells (Filley *et al.,* 2000). Mattos *et al.* (2000) suggested that the improvement effects of dietary fat on fertility of dairy cows was probably not a result of improvement of the energy status of the cow but that increased fertility could be due to effects of dietary fatty acids on the pituitary, ovaries and uterus.

Our results showed no significant change in serum copper and zinc in the cotton oil supplemented group compared with the control. Where there were significant increase in the level of serum total antioxidant in the supplemented group. Our results showed that cotton seed oil supplementation has antioxidative effect in postpartum cows. The same results observed by Moises (2006).

Cotton seed oil is rich in tocopherols (Duffy and Roberta, 2002), theoretically tocopherols isolated from cotton seed oil by (Emerson *et al.,* 1936). Vitamin E plays a role in preserving other vitamins (such as vitamin C and A) in body and protecting tissues against oxidation which might lead to inflammations and diseases Henmitt *et al.* (2003) revealed that antioxidant supplementation with vitamin E has been shown to have beneficial effects in preventing luteal phase deficiency and resultant increased pregnancy rate.

The significant higher conception rate observed in the dietary fat supplemented group compared to the control may be due to feeding dietary enrichment in linoleic and liolenic acid for postpartum cows which can influence fatty acids in plasma. The fatty acids in plasma can modulate the fatty acids in endometrial tissues and can modulate circulatory and intrauterine prostaglandins (E2 & F2). Prostaglandin E2 in uterine fluids has been previously reported to be associated with embryo survival and development (Chartrand *et al.,* 2003). Also (Lokesh *et al.,* 1992) reported that only the long chain fatty acids from the dietary fat were incorporated into liver lipids. The arachidonic acid in liver lipids was enhanced after supplementation of diets with sunflower oil. However the reduction in prostaglandins may be useful in reducing inflammatory responses mediated via prostaglandins. In addition Parmjit *et al.* (1992) suggested that high level of dietary fatty acids alter muscle membrane composition also result in alteration in glucose transport and the metabolism of muscle protein.

Zcheng *et al.* (2001) revealed that dietary poly unsaturated fatty acids (PUFAs) intake can reduce the endometrial capacity to produce prostaglandins and may therefore have implications for the control of luteolysis and other prostaglandin mediated events such as ovulation. Therefore, the role played by

PUFAs and eicosanoids in fertility show that feeding or infusing different types of fat with varying PUFA content to females can alter: the number and size of ovarian follicles, the ovulation rate, progesterone production by the corpus luteum and the timing of luteolysis.

Finally we can conclude that increasing level of fat percent by 2% cotton seed oil in the dry matter of the diet to post partum dairy buffaloes improve the reproductive response after synchronization using $PGF_{2\alpha}$.

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تأثير إضافة الدهون للعليقة على كفاءة البروستجلاندين المحقون للجاموس فى فترة ما بعد الولادة

أميمة مبروك ، رشاد حامد عثمان

اجريت الدراسة على عدد 40 جاموسة فى فترة ما بعد الولادة. تتراوح أعمار الجاموس ما بين 6-8 سنوات وسبق لها تحوصل الجسم الأصفر. قسمت الحيوانات الى مجموعتين الأولى عوملت كمجموعة ضابطة والثانية تم تغذيتها على 2% زيت بذرة القطن بالإضافى إلى مكون العليقة الأساسى واستمرت هذه التغذية حتى نهاية التجربة. جميع الجاموس الذى لوحظ به الجسم الأصفر تم حقنه بهرمون البروستاجلاندين فى كلا المجموعتين وتم تلقيحه بعد 72 ساعة من الحقن. تم فحص الحيوانات بعد 60 يوم من الحقن للتأكد من ثبوت العشار. تم أخذ عينات دم قبل الحقن وبعده بشهر وفصل مصل الدم من جميع الحيوانات لتحليل الجلوكوز والدهون الثلاثية والأحماض الدهنية والكوليستيرول وكذلك الزنك والنحاس وأيضا مجموع مضادات الأكسدة. أظهرت النتائج أن تركيزالجلوكوز فى الدم لم يتأثر معنويا باضافة الدهون فى العليقة لكلا المجموعتين. بينما ازداد تركيز الدهون الثلاثية والكوليستسرول فى الدم زيادة معنوية فى الحيوانات التى تمت تغذيتها بزيت بذرة القطن. كما تلاحظ أيضا الزيادة المعنوية فى دهون (palmitic, stearic. oleic, linolic and linolenic) في الحيوانات المغذاة على زيت بذرة القطن. ومن المشاهد فى استعراض النتائج أن عنصر الحديد والزنك لم يتأثر معنويا بين المجموعتين بينما مضادات الأكسدة الكلية معنويا قد ازدادت فى الجاموس الذى تغذى على زيت بذرة القطن. أما فيما يخص نسبة الإخصاب فهناك زيادة معنوية ملحوظة فى الجاموس الذى تمت تغذيته بزيت بذرة القطن اذا قورن بالمجموعة الضابطة.