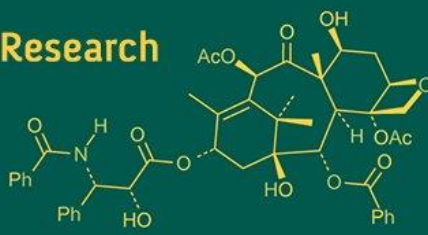
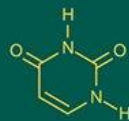
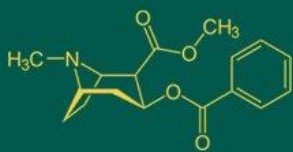


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CH Priyadarshini

Associate Professor,
Department of Veterinary
Biochemistry, College of
Veterinary Science,
Rajendranagar, Telangana,
India

P Eswara Prasad

Retired Professor and
University Head, Department
of Veterinary Biochemistry,
College of Veterinary Science,
Tirupati, Andhra Pradesh,
India

K Aswani Kumar

Department of Veterinary
Biochemistry, NTR College of
Veterinary Science,
Gannavaram, Andhra Pradesh,
India

D Nagalakshmi

Associate Dean, College of
Fisheries Science, Pebbair,
Telangana, India

D Madhuri

Associate Dean, College of
Veterinary Science,
Rajendranagar, Telangana,
India

Corresponding Author:**CH Priyadarshini**

Associate Professor,
Department of Veterinary
Biochemistry, College of
Veterinary Science,
Rajendranagar, Telangana,
India

Comparative effect of Ovsynch Protocol and dietary *Trigonella foenum graceum* seed supplementation on hormonal, biochemical and antioxidant profiles in postpartum anestrus buffaloes

CH Priyadarshini, P Eswara Prasad, K Aswani Kumar, D Nagalakshmi and D Madhuri

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Abstract

The present study was to study the comparison of biochemical parameters, antioxidant and hormonal profile of postpartum anestrus buffaloes treated with *Trigonella foenum graceum* seed powder to Ovsynch Protocol. A total of 16 postpartum anestrus buffaloes were selected and divided into two groups @ 8 buffaloes in each group. Group I (Control group) were treated with Conventional Ovsynch protocol (GnRH -PGF_{2α} - GnRH on 0-7-9th day respectively) and Group II were fed with *Trigonella foenum graceum* seed powder @ 200gm orally daily for 24 days. Blood sample was collected from jugular vein in specific vacutainers at an interval of 0, 8, 16 and 24th days for hormonal profile and 0th and 24th day for biochemical and antioxidant profile. Serum was isolated from blood and was analyzed for hormonal profile- progesterone and 17β-estradiol; biochemical parameters like glucose, protein, albumin, globulin, A/G ratio, total cholesterol and triglycerides; antioxidants viz lipid peroxidase, SOD and catalase. Serum progesterone in Group I showed significantly higher levels on 16th and 20th day whereas on 20th day, group II as well showed significant increase in progesterone levels. Serum 17 β-estradiol was increased significantly on 12th day in Group I and on 16th day in group II. The serum parameters like glucose, total cholesterol, triglycerides were significantly (P<0.01) lower, protein, albumin and globulin which were higher in group II, A/G ratio was non-significant. Serum lipid peroxidase levels were lower and SOD, catalase showed higher levels compared to Group I.

Keywords: Postpartum anestrus buffaloes, Ovsynch protocol, *Trigonella foenum graceum* seeds, hormonal profile, biochemical profile, antioxidant profile

Introduction

Postpartum anestrus remains one of the major reproductive constraints limiting productivity in dairy buffaloes. In India and other sub-tropical areas, postpartum anestrus in buffaloes may be due to several factors like negative energy balance, lack of antioxidants, hormonal imbalance, parity, anaemia etc. (Misra *et al.*, 2023) [11]. The prolonged anestrus period directly reduces calving rate, increases calving interval and contributes to economic losses in milk production and efficient reproduction. To combat postpartum anestrus in buffaloes, several hormonal therapies were applied that can restore ovarian cyclicity like GnRH, progesterone, insulin, PMSG (CIRD+PRID). The most effective and conventional applied hormonal protocol used in cattle and buffaloes is Ovsynch (Brahmanand *et al.*, 2023) [4]. The protocol uses sequential administration of GnRH and PGF_{2α} to synchronize follicular waves and induce ovulation, thereby shortening the anestrus period and enabling timed artificial insemination. Though effective, repeated hormonal use raises concerns about cost, residues, consumer perception and long term effects on reproductive physiology. Even prolonged or modified Ovsynch protocols can lead to reduced fertility, lower conception rates, and premature estrus, particularly if implemented without proper veterinary screening. (Sun S *et al.*, 2024) [19]. As an alternate to hormonal therapy, there are several non-hormonal herbal plants like *Murraya koenigii* (curry leaves), *Nigella sativa* (kalonji), *Abroma augusta* (Ulatkambal), *Saraca asoca* (Ashoka), *Trigonella foenum-graecum* (Methi), *Asparagus recemosus* (Shatavari), *Bambusa aruninacea*, *Leptadenia reticulata*, *Pergulacia daemia*, *Semecarpus anacardium*

cucumber, *Carica papaya*, *Courupita guianensis* and jute plants either alone or in combinations have been used to treat the anestrus with variable reaction on acceptance of estrus. (Chandra Shekhar Sarswat 2020) [5]. Due to antioxidant and anti-inflammatory compounds present in herbal plants, dietary intake of them will indirectly or directly ameliorate anestrus in postpartum buffaloes and also show other benefits like biochemical, antioxidant and anti-inflammatory effect. Further there are non-toxic. *Trigonella foenum graecum* seeds contain diosgenin, alkaloids, flavonoids, polyphenols that contribute to antioxidant, anti-inflammatory, antidiabetic, hypocholesterolemic activities. (Akhtar *et al.*, 2025) [2]. So, the present study is to compare effect of Ovsynch Protocol and dietary *Trigonella foenum graecum* seed supplementation on hormonal, biochemical and antioxidant profiles in postpartum anestrus buffaloes.

Materials and methods

A total of sixteen high yielding buffaloes after postpartum anestrus (>90 days) were selected after examination of follicular wave dynamics by routine trans - rectal ultrasonography and were divided into two groups; Group I was treated with Ovsynch protocol (GnRH -PGF_{2α} - GnRH on 0-7-9th day respectively) while group II were fed orally with *Trigonella foenum graecum* seeds powder @ 200gm daily once for 24 days. Blood samples were collected in specified vacutainers at an interval of 0, 8, 16 and 24th days

of feeding. Serum was isolated and analysed for hormonal profile viz Progesterone and 17β-estradiol, biochemical parameters like glucose, protein, albumin, globulin, A/G ratio, total cholesterol, and triglycerides; antioxidant parameters viz Lipid peroxidase, SOD and catalase.

Results and Discussion

The values of serum progesterone (ng/ml) and serum 17β-estradiol (pg/ml) were depicted in Table 1. Serum progesterone in Group I revealed significantly ($p<0.01$) higher than group II on 16th, 20th day and Group II showed increased levels on 20 and 24th day indicating that some animals had showed estrous on these days. This is similar to the findings of Rajkumar *et al.*, 2008¹⁴. The group II showed estrous in some animals might be due to diosgenin in *Trigonella foenum graecum* seeds which will promote ovarian folliculogenesis, hormone release, oocyte quality and prevent reproductive aging (Sirotkin *et al.*, 2019) [18]. The mean values of serum 17β-estradiol in Group I were significantly increased on 12th day whereas in group II the mean values were increased on 16th day indicating that the animals showed estrous in these days. Hind *et al.*, (2019) [7] observed that on feeding of fenugreek aqueous extract orally, the estrogenic activity was shown in vagina through an increase in epidermal cell number, tendency to keratinization, overexpression of progesterone receptors in the uterine tissue.

Table 1: Mean values of hormone levels (Progesterone and 17β-estradiol) of postpartum anestrus buffaloes 24 days treatment

Period (days)	Progesterone (ng/ml)		17β-estradiol (pg/ml)	
	Group I (Synthetic hormone)	Group II (FS)	Group I (Synthetic hormone)	Group II (FS)
0 th day	0.445±0.03	0.405±0.02	15.27±0.49	13.41±0.33
4 th day	0.491±0.03	0.450±0.02	15.71±0.48	13.87±0.33
8 th day	0.544±0.04	0.523±0.02	19.79±0.29	19.19±0.52
12 th day	0.824±0.18	0.589±0.02	24.82±5.48	21.68±0.65
16 th day	1.466±0.42	0.641±0.03	15.7 ±2.51	33.28±5.71
20 th day	2.269±0.66	1.144±0.32	16.00±1.79	29.19±7.29
24 th day	2.659±0.80	1.981±0.53	17.35±1.73	15.55±2.15
Overall Mean ± S.E	1.141±0.17	1.351±0.20	17.29±0.91	20.88±1.61

Table 2: Mean values of biochemical profile and antioxidant profile of postpartum anestrus buffaloes before and after 24 days treatment

Parameters	Group I		Group II	
	0 th day	24 th day	0 th day	24 th day
Biochemical profile				
Glucose (mg%)	56.47±2.50	43.59±2.18	61.69±1.71	36.40±1.66
Total Protein (g%)	5.46±0.092	7.24±0.081	5.45±0.116	7.31±0.053
Albumin (g%)	2.48±.037	3.11±0.021	2.58±0.077	3.22±0.077
Globulin (g%)	2.98±0.110	4.13±0.068	2.87±0.127	4.09±0.094
A/G ratio	0.84±0.044	0.75±0.011	0.97±0.094	0.89±0.034
Total cholesterol (mg%)	91.36±4.05	113.08±3.99	86.24±4.60	92.75±5.85
Triglycerides (mg%)	46.51±0.54	31.07±0.53	46.53±0.43	35.07±1.77
Antioxidant profile				
Lipid peroxidase (U/ml)	4.43±0.057	3.87±0.024	4.51±0.009	3.54±0.013
Superoxide dismutase (U/ml)	1.88±0.085	2.71±0.99	1.91±0.054	2.76±0.053
Catalase (U/ml)	1.46±0.42	1.71±0.035	1.48±0.047	2.20±0.046

The mean serum biochemical and antioxidant profile values were shown in Table 2. The mean serum glucose levels were significantly ($P<0.01$) lower in group II compared to group I indicating that glucose levels were reduced due to diosgenin in *Trigonella foenum graecum* seeds. Diosgenin will reduce serum glucose concentration and hepatic glucose-6-phosphatase activity and increase hepatic glucose-6-phosphate dehydrogenase (McAnuff M *et al.*, 2005) [10].

Decreased blood glucose levels may be due to insulin release that effects the peripheral tissue by promoting glucose uptake and metabolism, by attenuating the key enzymes of carbohydrate metabolism or by inhibiting hepatic gluconeogenesis (Saravanan *et.al.*, 2014) [15]. Hepatic enzymes like alanine aminotransferase, aspartate aminotransferase, gamma glutamyl transferase, lactate dehydrogenase and alkaline phosphatase lower the glycogen

level thus reduce serum glucose (Kaviarasam *et al.*, 2007) [8]. Increase in serum protein, albumin and globulin is observed in Group II compared to Group I. Similar findings were observed by feeding 200gm of fenugreek seeds in buffaloes by Abo El-Nor *et al.*, (2007) [1]; Kriar *et al.*, 2018 [9]. A/G ratio did not show any significant difference. Serum total cholesterol, triglycerides were reduced in group II compared to group I which might be due to the effect of diosgenin in *Trigonella foenum graecum* seeds. Diosgenin will increase ABCG5/G8 complex expression in liver and intestine which inhibit absorption of intestinal cholesterol by antagonizing Niemann-Pick C1-Like 1 (NPC1L1) function. (Dikkers *et al.*, 2010) [6]. Further it increases cholesterol 7 α -hydroxylase or cytochrome P450 7A1 (CYP7A1), carboxylesterase-1 (CES-1), and scavenger receptor class B type I (SRB1) levels in liver resulting in promoting reverse cholesterol transport, inhibit cholesterol absorption and eliminate cholesterol (Shi *et al.*, 2021) [16]. Diosgenin will upregulate sirtuin 6 expression and fatty acid transporters inhibiting liver from fatty acid absorption (Nie K *et al.*, 2023) [13]. Diosgenin enhancing biliary secretion and modify bile acid synthesis by regulating gut microbiome. It can decrease serum triglycerides and phospholipids and increased serum HDL concentration (Naskar and Mishra, 2017) [13]. Serum lipid peroxidase was reduced and superoxide dismutase activity, catalase activity increased in group II compared to group I. This is similar to the findings of Singaravelu *et al.*, (2018) [17], where the fenugreek treated animals showed increase in catalase, SOD and GSH compared to control positive group and significant decrease in TBARS value in treated group. The flavonoids and polyphenols in *Trigonella foenum graecum* seeds will accelerate antioxidant enzymes like catalase, SOD, glutathione peroxidase, glutathione reductase in the liver, skeletal muscle, cardiac muscle and brain (Baquer *et al.*, 2011) [3].

Conclusion

From the above study, postpartum anestrus in buffaloes on treatment with both conventional hormonal protocol and feeding with *Trigonella foenum graecum* seed powder showed estrous symptoms. Though Group I showed estrous earlier compared to group II, but in case of the biochemical profile and antioxidant profile, group II (FS treatment) has more beneficial effect compared to conventional hormonal protocol along with exhibiting of estrous cycle.

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