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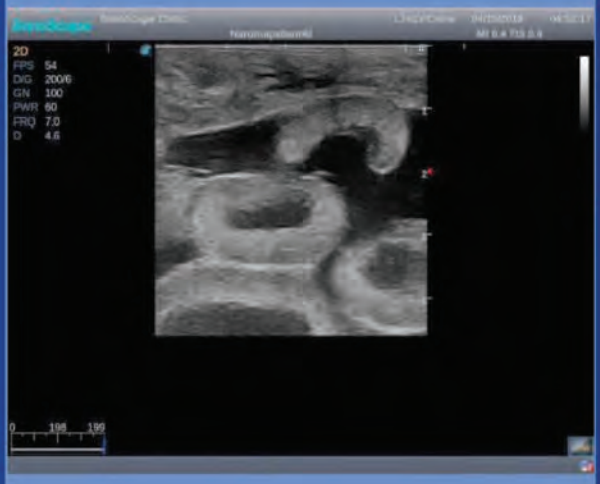

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Full length articles

Short Communications

Full Length Articles

Supplementation of fish meal and exogenous administration of COX-2 inhibitor on conception rate in repeat breeding Jersey crossbred cows

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ABSTRACT

The study was conducted on repeat breeding cows at Large Animal Gynaecology Ward, Department of Clinics, Madras Veterinary College, Chennai and some private cattle farms in and around Koduvalli region, Tiruvallur district, Tamil Nadu. In experiment I, Jersey crossbred cows (n=31) synchronized by $PGF_2\alpha$ and inseminated on exhibited estrum. The cows were randomly divided into five groups viz. Group I (control, n=6), group II (n=7,100mg), group III (n=6, 150mg), group IV (n=6, 200mg) and group V (n=6, 250mg) and supplemented with fish meal on day 13, 14 and 15 of post insemination in addition to their routine feed. Pregnancy diagnosis was carried out by rectal palpation on day 60 of post insemination. In experiment II, twelve repeat breeding Jersey crossbred cows were selected and synchronized using $PGF_2\alpha$ followed by timed insemination. On day 10 of post estrus, rectal examination was performed to identify the corpus luteum and randomly divided into two group viz. Group I and Group II (control). Group I cows were injected with Meloxicam @ 0.5 mg/ kg body weight through intramuscularly on day 13 to 15 post-insemination. The conception rate in experiment I as in Group I, group II, group III, group IV and group V are 16.67%, 71.43%, 66.67%, 66.67% and 50.0%, respectively. The conception rate in meloxicam treated animal was 66.7% and in control animal was 33.3%. The fish meal treatment and Cox-2 inhibitor in repeat breeding crossbred cows had improved the conception rate. Further, the supplementation of 100mg of fish meal in repeat breeding cows has shown higher conception rate as compared to other groups in experiment II. Hence, it may be used in field condition for enhancement of conception rate, however, further studies in larger population is warranted.

Key Words: Cattle, Fish meal, COX2 inhibitor, Conception rate

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INTRODUCTION

Though the fertilization rates are as high as 80-95%, the embryonic mortality is one of the major causes for the poor reproductive efficiency since 40-53% of embryos were lost–between 8 and 16 days of pregnancy in cattle (Binelli *et al*., 2009). Recently, $PF_2\alpha$ synthesis inhibitors have been used to modulate endometrial $PGF_{2\alpha}$ production either by manipulating oxytocin receptor system or subsequent $PGF_2\alpha$ synthesizing enzymatic machinery especially COX-2 and PGF Synthase pathway to improve the fertility rate in bovine. Administration of oxytocin receptor antagonist between days 12 and 20 of the cycle has been reported to suppress the increase in PGFM concentration and delays luteal regression in cyclic goats (Homeida and Khalafalla 1987). Similarly, nonspecific COX inhibitor (Flunixin and meloxicam) has been shown to inhibit luteolysis and extended the estrous cycle in cycling heifers (Aiumlamai *et al*., 1990). Administration of COX inhibitors viz. flunixin meglumine and ibuprofen prior to embryo transfer has been reported to increase pregnancy rate (10 to 25%) through inhibition of uterine $PGF_2\alpha$ release in cattle (McNaughtan *et al*., 2002). It also decreased embryonic loss caused by stress on day 14 post-insemination in beef cows (Merrill *et al*., 2003). Recently, administration of flunixin on day 15 and 16 post-insemination increased conception rate as compared to control (69.2% vs. 46.2%) in Holstein heifers (Guzeloglu *et al*., 2007). COX-2 is the primary isoenzyme involved in the endometrial production of $PGF_2\alpha$ rather than COX-1. Moreover, COX-1 is a constitutive enzyme involved in normal physiological process and its inhibition may affect the normal physiological functions in the body. Hence, selective COX-2 inhibitor would be more effective in preventing $PGF_2\alpha$ synthesis and enhancing fertility as compared to non-specific COX inhibitors.

Shukla (2006) reported that administration of COX-2 inhibitor (meloxicam) on day 15 and 16 of estrous cycle increased the cycle length as compared to control in buffalo. Therefore, administration of drugs like selective COX-2 inhibitor and PGFS inhibitor may inhibit $PGF_2\alpha$ production from the endometrium and enhance embryo survival thus fertility. Recently, emphasis has been given on the COX-2 inhibitor, feeding of polyunsaturated fatty acid for the enhancement of embryonic survival and thus augmentation of fertility. Information pertaining to these drugs and inhibitors on embryonic survival and fertility are as such meager in bovine. The attainment of the objectives proposed in the present study would, therefore, be helpful to develop package of practices for augmentation of fertility and thus productivity of bovine.

MATERIALS AND METHODS

Place of study: The study was conducted on repeat breeding cows at Large Animal Clinic Gyneacology Ward, Department of Clinics, Madras Veterinary College, Chennai and some private cattle farms in and around Koduvalli region, Tiruvallur district, Tamil Nadu.

Experimental Design: In Experiment I, the repeat breeding cows were selected through infertility camp near Koduvalli and Red Hills region, Tiruvallur district,

Tamil Nadu. Forty repeat breeding cows were selected for this study and screened for subclinical endometritis. Nine repeat breeding crossbred animal out of 40 were identified as positive for subclinical endometritis through White side test and those animals were not included for the study and remaining 31 animals were selected for the study. Experimental cow subjected to detection of estrus after synchronization by using PGF2α by visual observations and rectal palpation. The cross-bred Jersey cows detected in estrus were inseminated using frozen semen straws and randomly divided into five group viz. Group I (control, n=6), group II (n=7, fish meal treatment, 100mg), group III (n=6, fish meal treatment, 150mg), group IV (n=6, fish meal treatment, 200mg) and group V (n=6, fish meal treatment, 250mg). All experimental cows except control were supplemented fish meal with 50g of jaggery on 13, 14 and 15 of post insemination in addition to their routine feed. In experiment II, twelve repeat breeding cows were selected for the study. Experimental cows were subjected to detection of estrus after synchronization by using PGF2α by visual observations and rectal palpation. Cows were detected in estrus inseminated using frozen semen straws. Each animal was palpated per rectum on day 10 post estrus for the presence of corpus luteum and randomly divided into two group viz. Group I (Inj. Meloxicam treatment) and group II (control). Animals of group I were injected with Meloxicam (Melonex TM, Intas Pharmaceuticals Ltd., Ahmedabad, India) @ 0.5 mg per kg body weight per day intramuscularly on day 13 to 15 post-insemination. The animals of group II were untreated and kept as control. Blood samples were collected from all experimental animals by jugular venipuncture on day 13, 15, 18, 21 and 24 post-insemination. The serum was separated and stored at -20° C until progesterone estimation. The pregnancy diagnosis was carried out through per-rectum on 60 days post insemination. The concentration of progesterone was estimated using the diagnostic I125 kits supplied by Immunotech, France. For progesterone analytical sensitivity of the kit was 0.05ng/ml; the intra-assay and inter-assay coefficient of variation 5.8% and 9.0%, respectively. The pregnancy diagnosis was carried out through per-rectum on 60 days post insemination.

Statistical analysis: The statistical analysis was carried out using SPSS version 13.0 for windows. The data for progesterone profile were analyzed within groups by one-way ANOVA.

RESULTS AND DISCUSSION

The conception rate in experiment I as in Groups I, II, III, IV and V are 16.67, 71.43, 66.67, 66.67 and 50.0%, respectively (Table 1). Among that feeding of fish meal about 100g gives more conception rate. The results indicate that the feeding of PUFA during critical period can be used to inhibit secretion of endometrial PGF2α which occurs due to delay or insufficient IFN-τ secretion by embryo or decrease in response of endometrium to the IFN-τ, resulting in failure of Maternal Recognition of Pregnancy (MRP). *In vitro* studies revealed uterine tissues when incubated in medium only (M) or media supplemented with fatty acids viz. eicosapentaenoic

(20:5omega3; EPA), docosahexaenoic acids (22:6omega3; DHA) or linoleic acid (C18:2omega6; LIN) revealed luteotrophic PGE_2 release from pregnant endometria was higher than from non pregnant endometria, while $PGF_2\alpha$ concentrations were similar. Polyunsaturated fatty acids like EPA or DHA inhibit secretion of $PGF_2\alpha$ from bovine endometrial cells in vitro. EPA and DHA exert their regulatory effects as alternative substitutes that reduce the lipid pool of arachidonic acid in the endometrium. High concentrations of EPA and DHA has been reported in Menhaden fish meal and supplementation of lactating dairy cows with Menhaden fish meal for 25 days reduced PGFM plasma concentrations in response to an estrogen injection followed by an oxytocin injection on day 15 of a synchronized estrous cycle.

Table 1. The effect of selective COX-2 inhibitor (fish meal) on conception rate in repeat breeding crossbred cows

S. No	Attributes	Group I (Control)	Group II	Group III	Group IV	Group V
1.	Animals treated	6	7	6	6	6
2.	Animal Responded for estrus synchronization ($PGF_2\alpha$)	6	7	6	6	6
3.	Onset of estrus following estrus synchronization (h)	58.00	58.43	60.67	58.34	61.00
4.	Duration of estrus	19.42	20.57	22.57	18.57	18.00
5.	Duration of treatment (days)	--	13,14 and 15 Post AI (3 days)	13,14 and 15 Post AI (3 days)	13,14 and 15 Post AI (3 days)	13,14 and 15 Post AI (3 days)
6.	Conception rate (%)	1/6 (16.67)	5/7 (71.43)	4/6 (66.67)	4/6 (66.67)	3/6 (50.00)

The analysis of experiment II has shown that the meloxicam treated animals are having higher conception rate 66.7% as compared to control group 33.3% (Table 2). Among COX inhibitors potential candidates capable of inhibiting uterine $PGF_2\alpha$ release are non-steroidal anti-inflammatory drugs (NSAIDs). NSAIDs act by competitive inhibition of COX enzyme either non-selectively through both COX-1 and COX-2 isoforms *i.e.* flunixin, ibuprofen or specifically through COX-2 isoform *i.e.* celecoxib, rofecoxib, valdecoxib, and also through preferentially inhibiting COX-2 viz. meloxicam, nimusulide etc. Research has shown that intensive parenteral administration of flunixin is able to postpone luteolysis and prolong the estrous cycle in heifers. The present study was in agreement with studies by Rajkumar *et al*., (2010) who observed meloxicam as COX-2 inhibitor improved pregnancy rates in buffaloes post-Artificial Insemination. Further, Ladol *et al*. (2010) who also reported 60 per cent conception rate in repeat breeding buffaloes treated with meloxicam @ 0.5 mg/kg, body weight, intramuscularly on days 13, 14 and 15 post AI whereas it was 25 per cent only in control.

Table 2. The effect of selective COX-2 inhibitor (inj. Meloxicam) on conception rate in repeat breeding crossbred cows

S. No	Attributes	Group I (Inj. Meloxicam, n=6)	Group II (Control, n=6)
1.	Animals treated	6	6
2.	Duration of treatment (days)	3	3
3.	Animal Responded for estrus synchronization ($PGF_2\alpha$)	6	6
4.	Onset of estrus following estrus synchronization (h)	59.67	60.34
5.	Duration of estrus	18.34	20.33
6.	Conception rate (%)	**4/6 (66.7)**	**2/6 (33.3)**

The average progesterone concentration estimated on 13, 15, 18, 21 and 24 post-insemination on individual pregnant Jersey crossbred cow basis varied from 5.32±0.04 to 6.41±1.02; 4.87±.20 to 6.72±0.05; 3.87±0.25 to 5.93±0.60; 5.04±0.31 to 6.41±0.10 and 5.32±0.20 to 6.43±0.14 for groups I, II, III, IV and V, respectively. The present results indicate that the progesterone values are getting increased significantly (P>0.5) after day 20 of pregnancy and shows increasing trend as the gestation advances in pregnant cows. However, the value of progesterone in non- pregnant cows is getting decreased on day 15 onwards and reaches in basal level on day 20 of post cycle. These results also support the establishment of pregnancy after feeding of fish meal to the cows which are affected with repeat breeding syndrome. As a conclusion the selective COX-2 inhibitor and fish meal treatment in repeat breeding cross-bred cows had improved the conception rate. Hence, it may be used in field condition for improvement of conception rate, however, further studies in larger population is warranted.

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Low haemoglobin density as an anaemic indicator in canine babesiosis

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ABSTRACT

Low Hemoglobin Density (LHD) is related to functional iron availability and hemoglobin content. To assess the use of LHD as an anaemic indicator in canine babesiosis, 108 dogs with symptoms of canine babesiosis were selected and screened for disease by polymerase chain reaction. Blood samples were collected for estimating haematological variables, serum iron, TIBC, UIBC and LHD. Based on the haemoglobin, PCV and erythrocyte count the animals were grouped into anaemic and non-anaemic populations. LHD% was higher in the anaemic population (P<0.001) and it was negatively correlated to haemoglobin, packed cell volume, erythrocytes, MCHC and serum iron. LHD% was highly depend on serum iron (P<0.001). Receiver operating characteristics (ROC) curve analysis revealed LHD was 100.00% specific and 80.43% sensitive in identifying the functional iron deficient anaemia with cut-off value of 1.83%. This study reveals that LHD is a cost-effective parameter for identifying the functional iron deficient anaemia in canine babeisosis.

Key Words: Anaemia, Canine babesiosis, Iron deficiency, LHD.

INTRODUCTION

Anaemia is an imperative clinical sign of many systemic, infectious and parasitic diseases. Evaluating the cause and type of anaemia will provide adequate information about patho-physiology of diseases (Aird, 2000; Morrison, 2005). Complete blood count, haemoglobin level, packed cell volume, and erythrocyte indices were used for diagnosing anaemia (Vieth and Lane, 2017). Apart from these normal haematological parameters estimation of micro-minerals, like iron can help in diagnosing different types of anaemia such as iron deficiency anaemia, anaemia due to chronic diseases, anaemia of inflammatory disease and iron overload anaemia (Lieu *et al*., 2001; McCown and Specht, 2011). It also aids in characterising the anaemia into regenerative or non- regenerative type (Mitchell and Kruth, 2010). Serum iron, serum total iron binding capacity (TIBC), serum ferritin and per-cent transferrin saturation (%TSAT) were normally used parameters to measure the iron status of animals (Wians *et al.,* 2001). Among these parameters serum ferritin concentration has limited use in veterinary practice because of its species-specificity and limited assay availability (Schaefer and Stokol, 2015).

Currently available iron tests were influenced by acute phase response and are also expensive (Bovy *et al*., 2007; Brugnara, 2003; Coyne, 2006; Mast *et al*.,

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2002). To evade these problems, Urrechaga (2010) proposed a parameter, Low hemoglobin Density (LHD %) derived from the mathematical sigmoid transformation of mean cell hemoglobin concentration (MCHC). MCHC is a traditional estimator of iron availability preceding 90–120 days. As like MCHC, LDH is linked to iron availability and haemoglobin content of erythrocyte. The clinical reliability of LHD as an iron availability marker for erythropoiesis have been documented early (Urrechaga *et al.,* 2010; Urrechaga *et al.,* 2012).

Canine babesiosis is a hemoprotozoan parasitic disease caused by apicomplexan parasites of the genera Babesia, which is characterized by haemolyticanaemia, jaundice, lethargy, pyrexia and haemoglobinurea (Solano-Gallego and Baneth, 2011). *Babesia gibsoni* (small form) and *Babesia canis* (large form) are the two important species reported in natural infections in dogs in India. The clinical presentation is diverse and ranges from transient anorexia to a complex syndrome in which multiple organ systems are affected. Anaemia and thrombocytopenia are the most common haematological abnormalities observed in canine babesiosis. Initially, anaemia will be mild, normocytic and normochromic, as the disease progresses it becomes macrocytic, hypochromic and regenerative (Lobetti, 2003).Even though anaemia is an important clinical sign of babeisosis, some of the infected dogs did not show the clinical signs of anaemia and also noalterations in their haematological parameters like erythrocyte count, haemoglobin level and erythrocyte indices (Zamokas *et al.,* 2014). Considering these facts we aimed to identify the changes in LHD among babesia infected dogs (anaemic and non-anaemic groups) and also to know about the ability of LHD in diagnosinganaemia in these dogs.

MATERIALS AND METHODS

A total of 108 dogs (47 males and 61 females), aged 2 months to 13 years, brought to Madras Veterinary College Teaching Hospital and Blue Cross of India, Chennai with clinical signs such as pyrexia coupled with haemoglobinuria, tick infestation, lymphadenopathy and lethargy were included in the study group. PCR was done to confirm the presence of *Babesia*infection. Blood samples were collected from all animals on the first day of presentation. Based on the traditional anaemic markers levels (Haemoglobin, PCV, erythrocytes), the affected animals were grouped into anaemic (n = 58, Male - 25, Female – 33) and non-anaemic group (n = 50, Male - 22, Female – 28). The haematological parameters like Haemoglobin (Hb) by acid hematin method, packed cell volume (PCV) by microhematocrit method, erythrocyte count (TEC) by hemocytometer method were recorded and erythrocyte indices calculation were done as per the method given by Coles (1986). Serum iron, transferrin iron binding capacity (TIBC) and unsaturated iron binding capacity (UIBC) were estimated from all the samples by Ferrozine method using colorimetric kit supplied by Coral clinical systems, Goa, India- 403202. LHD% was calculated from the MCHC values using the formula described by Urrechaga (2010).

$$LHD\% = 100 * \sqrt{1 - (1/(1 + e^{1.8(30 - MCHC)}))}$$

Unpaired Student'st-test was used to identify the statistical difference of estimated parameters between these groups. Relationship between LHD and other parameters was estimated by Pearson correlation. Linear regression model was used to identify the dependency between LDH and serum iron. Receiver operating characteristics (ROC) curve analysis was used to identify the anaemic diagnostic cut-off value, sensitivity, specificity, area under curve (AUC) and Youden's index of LHD. All analyses were performed using SPSS IBM Version 23 software.

RESULTS AND DISCUSSION

Serum iron was significantly reduced and LHD, TIBC and UIBC were significantly ($P<0.001$) elevated in the infected dogs with anaemia (Fig. 1). In the current study, reduction in the serum iron concentration of anaemic population indicates the ineffective erythropoiesis leading to anaemia which was evidenced as decreased haemoglobin and erythrocytes count. Because during iron deficiency, the impairment in the haemoglobin synthesis causes reduction in circulating erythrocytes level and they will be hypochromic in nature (Brugnara, 2003). LHD is a marker for hypochromasia, which reflects the period of iron deficiency during erythropoiesis (Urrechaga, 2016). Elevation of LHD % in the anaemic group also reveals the on-going ineffective erythropoiesis which accelerates the prevailing anaemic condition. Increase in the transferrin iron binding capacity of the anaemic group indicates the effective binding capacity of transferrin molecule to increase the utilization of limited functional iron available in the circulation. Considering these variations in the anaemic and non-anaemic group of babeiosis dogs, LHD can also indicate the anaemic status as like other standard anaemic parameters like haemoglobin, erythrocyte and packed cell volume.

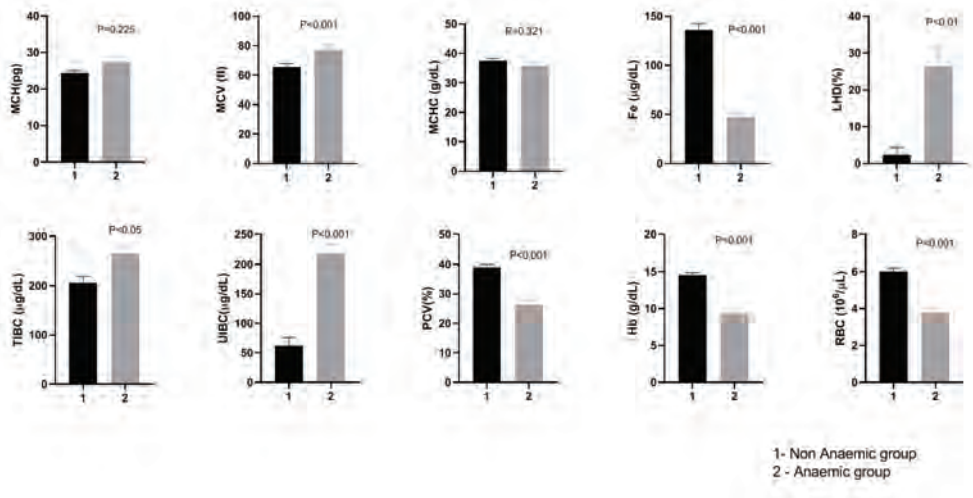

Figure 1: Results of unpaired t test for different parameters analysed between anaemic and non-anaemic groups of dogs with babesiosis.

Pearson correlation between LHD and other standard anaemic parameters revealed the significant (P<0.01) negative relationship of LHD to haemoglobin, packed cell volume, erythrocytes, MCHC and serum iron (Fig. 2). This indicates the increase in LHD will cause decrease in haemoglobin, packed cell volume, erythrocytes, MCHC and serum iron. Reduction in functional iron reserve for erythropoiesis may be a reason for this negative relationship between LHD and other anaemic parameters. Hence, evaluation of the LHD will be an added advantage in diagnosing different types of anaemia. In canine babesiosis, the serum iron level is not a static one and its concentration differs according the severity of anaemia (Lobetti, 2003). Thus, LHD evaluation along with other anaemic parameters will provide information about the functional iron reserve of the affected animal.

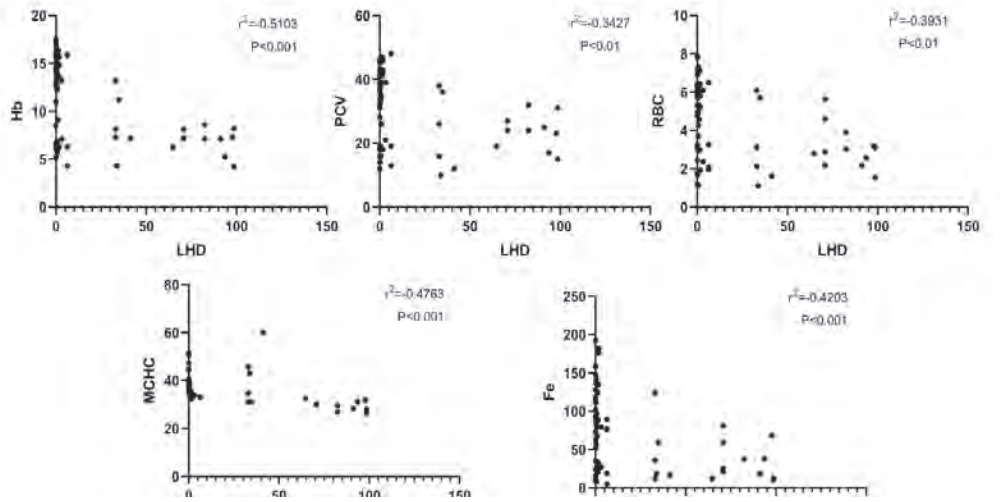

Figure 2: Results of Pearson correlation of LDH to other parameters analysed in the study.

Linear regression analysis revealed significantly (P<0.001) high dependency between LHD and serum iron (Fig. 3). This provides information for predicting the functional serum iron level by simple mathematical sigmoid transformation of MCHC. Currently available iron tests are influenced by acute phase response, expensive, species specific and limited in availability; which makes them ineffective in veterinary practice (Schaefer and Stokol, 2015). Use of this LHD % as an indicator of iron status in different clinical conditions can evade the above said problems.

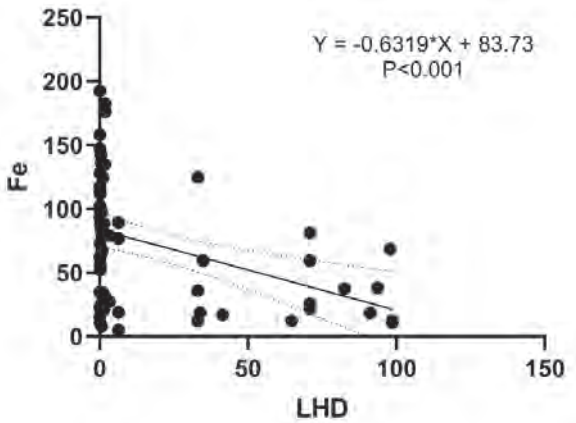

Figure 3: Linear regression model indicating the relationship between LHD and serum iron concentration.

Receiver operating characteristics (ROC) curve analysis identified the anaemic diagnostic cut-off value as >1.83% at the confidence interval of 84.3 and 98.2% (P<0.001) with 100.00% specificity and 80.43% sensitivity. Area under curve (AUC) of LHD was about 0.935 and the Youden's index was 0.804 (Fig. 4). This was similar to diagnostic cut-off value of LHD% for iron status indication in humans (Urrechaga *et al.*, 2010). In the current study, LHD was more specific in identifying the healthy population without anaemia and also it indicates that the increase in the LHD% more than the cut-off range will predispose the animal to functional iron deficient anaemia. Previous reports in humans also suggests that the LHD can be used as an indicator for the functional iron status and it can be used in the early investigation of anaemia(Urrechaga *et al.*, 2010; Urrechaga *et al*., 2012; Urrechaga, 2016).

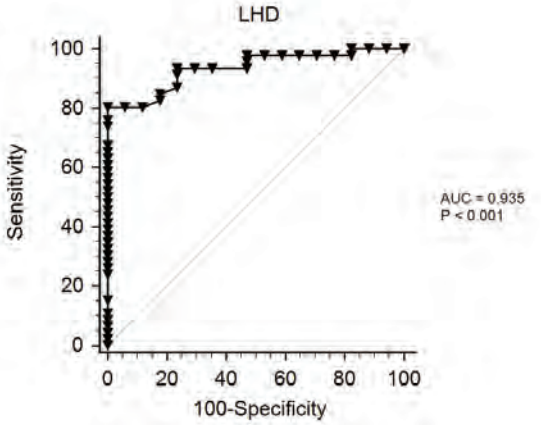

Figure 4: Receiver operating characteristics (ROC) curve analysis with area under curve for identifying the diagnostic sensitivity and specificity of LHD.

In the current study it was identified that use of LHD in conjunction with standard blood cell counts and iron parameters enable the more rapid and accurate diagnosis of anaemia in the babesiosis affected animals. Cost- effectiveness of LHD than other iron status indicator tests normally employed in veterinary practice is an added advantage to this parameter. First-hand information obtained from this parameter will be extremely useful to the veterinarians in diagnosis and monitoring the treatment response when the LHD% is above 1.83 %. Since this is a first study to evaluate the use of LHD as anaemic indicator in veterinary clinical medicine, still more researches are warranted to support this finding in different clinical conditions.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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Comparison of endocervical and endometrial cytology to diagnose sub-clinical endometritis in repeat breeding cows

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ABSTRACT

Repeat breeding due to sub clinical endometritis (SCE) is often diagnosed by cytological examination and the effect of endocervical inflammation (ECI) on increased hazards of pregnancy is inadequately investigated. In this study, endocervical cytology was compared with the golden standard test 'endometrial cytology' to detect SCE as a cause of repeat breeding in cows. Influx of Neutrophils and endocervical inflammation will reflect the status of endometrium and may indirectly reflects the ongoing sub clinical form of endometrial inflammation. Hence, this study was aimed at fixing threshold for PMN in endocervical cytology and comparing the same with golden standard technique. Repeat breeding cows associated with endocervical inflammation with >8% of neutrophils during standing estrus in endocervical cytology will have increased hazards of pregnancy. Our study suggested that a moderate concordance/association between ECI and endometrial inflammation (EDI) in repeat breeding cows, these findings were based on the endocervical and endometrial cytology. Based on the endocervical cytology examination 85% of repeat breeding cow posses EDI (SCE) with a diagnostic sensitivity of 86% and showing moderate clinical acceptability for application of endocervical cytology as diagnostic aid to detect SCE in repeat breeding cows.

Key Words: endocervical cytology, subclinical endometritis, repeat breeding, cross bred cows

INTRODUCTION

Repeat breeding due to subclinical endometritis (SCE) often creates inappropriate uterine environment which seriously impedes the fertilization process and embryonic growth (Sheldon *et al*., 2009). Vagaries of studies have demonstrated various diagnostic techniques to detect SCE with varying degree of accuracy and clinical applicability (Barlund *et al.,* 2008; Sheldon *et al*., 2008 and deBoer *et al*., 2014).

Cows with SCE were diagnosed by the uterine lavage based endometrial cytology harvesting bovine endometrial cells and PMNs with uterine lavage is considered to be a consistent golden standard technique and reflects the endometrial status. PMN cut –off of more than 3% in endometrial cytology beyond 35 Days In Milk (DIM) was designated as SCE or cytological endometritis (Galvão *et al*., 2011; El-Amrawi and El-Karim, 2019). Ahmadi *et al*., (2016) demonstrated the

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collection of endocervical mucus (ECM) in cows and their easy handling in contrast to uterine lavage (UL).It was hypothesized that the changes in endometrial cytology during the diseased condition will be reflected in the cervical cytology. Hence the present study was envisaged to investigate the efficiency of ECM in comparison with UL to detect SCE in cows.

MATERIALS AND METHODS

Experimental Animals

The present study was conducted at Gynaecology and Obstetrics section, Veterinary Clinical complex, Veterinary College and Research Institute, Tirunelveli. Pluriparous cows presented with an anamnesis of infertility were subjected for gynaeco-clinical and ultrasonographic examinations. A total of 28 crossbred cows with repeat breeding syndrome were selected for the study.

Assessment of PMN cells in endocervical mucus

During the observed oestrus the ECM was collected from the cows as described by Adnane *et al*. (2017). The vulva was cleaned with 0.1% potassium permanganate solution and a sterile uterine sheath attached to a 20 ml syringe was fixed at the endocervix by recto-vaginal method and ECM was aspirated. The mucus was smeared on glass slides and stained using Leishman stain for cytological evaluation as described by Adnane *et al*. (2017) with slight modification. Briefly the aspirated and agitated mucus was smeared on the grease free glass slide and air dried before staining, the dried smear was then stained by using Lieshmann stain . A total of 100 nucleated cells were counted in 10 random high-power fields (10 cells per HPF), and PMN cells ratio were averaged by HPF100 method (Adnane *et al*., 2018).

Assessment of PMN cells in uterine lavage

After collection of ECM, UL was collected from the same animal by adopting a slight modification (i.e low volume uterine lavage technique) in the collection procedure described by Cheong *et al*. (2012). Ten millilitre of sterile 0.9% normal saline solution was infused into the uterus using guarded sterile uterine sheath attached to a 20 ml syringe. The uterus was gently massaged and the fluid was aspirated. The recovered fluid was centrifuged at 2500 xg for 10 min. The sediment was smeared on the glass slides and stained using Leishman stain. A total of 100 nucleated cells were counted in 10 random high-power fields (10 cells per HPF), and PMN cells ratio was averaged by HPF100 method (Melcher *et al*., 2014).

Keeping UL PMN ratio of > 3% as cut off value for the detection of SCE (Fischer *et al.*, 2010), animals were categorized as with or without SCE. Based on these criteria the PMN % in ECM and UL were compared and analyzed.

Statistical analysis

The results obtained from enumeration of PMN from ECM was compared with UL for its diagnostic sensitivity, specificity, Lin's concordance correlation coefficient and Bland-Altman plot to assess the agreement between two assays and their concordance will be interpreted according the criteria established by Smith (2009).

RESULTS AND DISCUSSION

The mean percentages of PMN cells in animals with and without SCE were presented in Table 1. Based on the 'gold standard' of UL PMN cut-off ratio, 24 (85.71%) out of 28 repeat breeder cows were found to be positive for SCE.

The average PMN in the SCE positive animals was 5.90 (Tab.1) which was above the cut off value of 3 per cent (Sheldon *et al*., 2009 and Madoz *et al*., 2013). Influx of PMN into the endometrial mucosa of cows above threshold values is always associated

with acute active endometrial inflammation and conception failure often leads to repeat breeding in cows (Turner *et al*., 2012).

Examination of ECM cytology revealed that the entire entire animals positive for SCE (as per UL cytology) had a clear differentiation in the mean percentage of PMN cells (8.20 ± 0.82) (Fig.1) when compared with those animals without SCE (3.75 ± 1.3) (Fig.2). The finding suggested that the endometrial cytological changes during SCE can be extrapolated to the endocervical cytology and hence a positive correlation could be ascertained between the two genital environments.

It was observed that the diagnostic sensitivity of endocervical cytology was 86% and specificity of 75% and Lin's correlation coefficient of 0.61 with moderate agreement ($p<0.05$). Our findings imply that appearance of PMN above threshold values in endocervical cytology of repeat breeding cows demonstrates a moderate concordance with occurrence of SCE (Table 1). Hence, our study found that the presence of >8% PMN in endocervical cytology can detect SCE in repeat breeding cows and such cows will have increased hazards of pregnancy failure. The mean difference in PMN's of endocervical and endometrial region was plotted by the Bland-Altman graphical method and the plot revealed moderate concordance between inflammatory status of the endometrial and endocervical region. The Bland-Altman plot method indicated a mean difference in PMN% between endometrial and endocervical specimens of 5% and 8% respectively with 95% limits of agreement of -14 to 12 % (Fig.3). This plot suggests that the difference in the inflammatory status of endocervix and endometrial mucosa can be compared with acceptable magnitude of mean difference between assays.

It can be concluded that the cows with endocervical cytology of above threshold value of > 8% PMN could be considered positive for SCE. By virtue of easy collection and handling, more efficient method requiring less expertise when compared to UL for collection and processing of samples, endocervical cytology can be used as a diagnostic criterion to detect SCE in repeat breeding cows.

Table: 1. Mean±SE values of PMN% in repeat breeder cows with or without SCE

	(%PMN)	
	SCE Negative (n=4)	**SCE Positive (n=24)**
Uterine Lavage (UL)	1.75±.025	5.90 ± 0.80
ECM	3.75 ±1.3	8.20 ± 0.82

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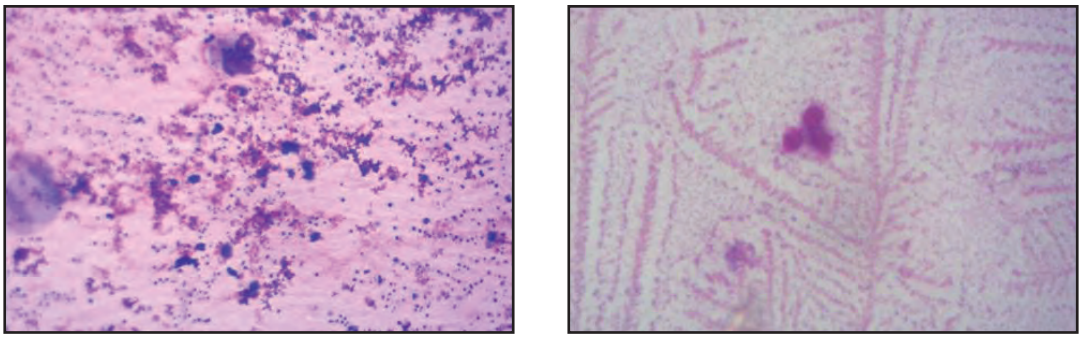

Fig.1. PMN- Endocervical cytology

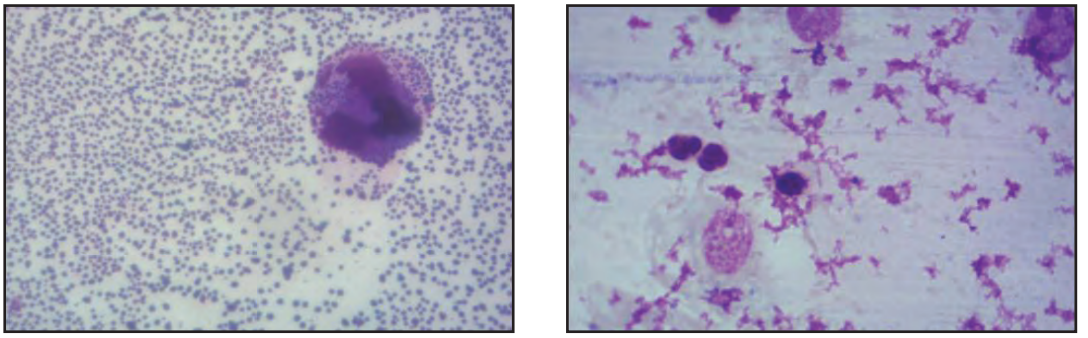

Fig.2.PMN –Endometrial cytology

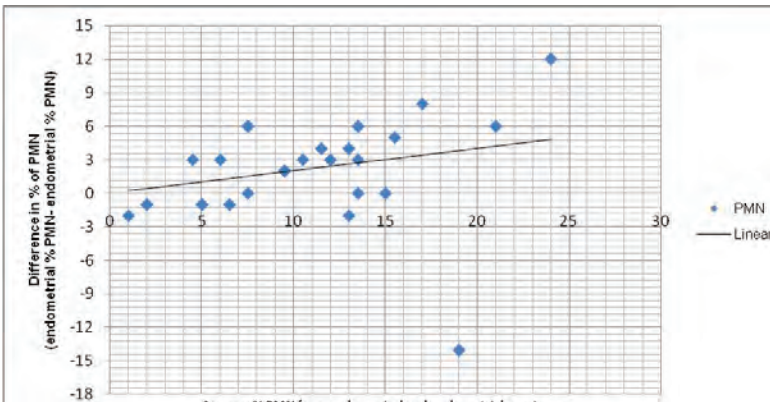

Fig.3. Bland-Altman plot of the data of the percentage of neutrophils (%N) in endometrial and endocervical specimens for each cow (n=24)

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Assessment on the incidence of aflatoxin B_1 in composite cattle feed and raw materials in India

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ABSTRACT

Composite cattle feed samples and raw materials like maize, wheat, sorghum, broken rice, pearl millet, groundnut cake, cotton seed cake, mustard cake, soyabean meal, rapeseed meal, sunflower meal, guar meal, safflower meal, deoiled rice bran and wheat bran were collected from various parts of India for a period 5 years and evaluated for aflatoxin B_1 level by screening through ELISA and confirmation through HPLC. Among the 482 composite cattle feed samples, only 68.5per cent were having aflatoxin B_1level below the permissible level (below 20 ppb). Higher incidence of aflatoxin B_1 was recorded in west zone than east, north and south zones during monsoon period. However, it was observed that incidence of aflatoxin B_1 at concentration beyond 100ppb was relatively higher in eastern zone both during monsoon and non-monsoon period than the west, north and south zones. Among the energy source, maize had the highest (7.8 per cent, 15 out of 193 samples tested) prevalence of alarming levels of aflatoxin (>100ppb) and 41.5 per cent (80 samples) in the range of 21 to 100 ppb of aflatoxin B_1. Only 50.8 per cent (98 samples) were within permissible level (<20 ppb). It was observed that the presence of aflatoxin $B_1$was more in maize during monsoon in south zone. Only 47.2 per cent of groundnut cake (118 samples) were within permissible level of <20 ppb of aflatoxin B_1. In west zone, highest sample profile (15.18per cent) and zone-wise distribution of 45.45per cent was recorded in the samples of groundnut cake having aflatoxin B_1 at alarmingly high level of more than 100 ppb. Overall data reveals that monsoon favours development of aflatoxin B_1 more during monsoon.

Key Words: Cattle feed, Cakes, Meals, raw materials, Aflatoxin B_1, ELISA, HPLC.

INTRODUCTION

Aflatoxins are potent toxic, carcinogenic, mutagenic, immunosuppressive agents, produced as secondary metabolites by the fungus *Aspergillus flavus* and *A. parasiticus*on variety of food products.

Among18 different types of aflatoxins identified, Aflatoxin B_1 is found in the highest concentration and most potent toxin. Aflatoxins are found in many countries, especially in tropical and subtropical regions where conditions of temperature and humidity are optimum for growth of the molds and for production of the toxin. Eliminating them totally from feed and feed ingredients is not an easy task (Azarakhsh *et*

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al., 2011). Aflatoxin B_1 is naturally present in several important feedstuffs like peanut, maize, cotton seed and damaged grains. (Khan *et al.*, 2011). These ingredients are widely used as raw materials in formulation of balanced cattle feed. An important feature in dairying is the fact that aflatoxin B_1 shows a significant carry-over into the milk in its hydroxylated metabolized form of aflatoxin M_1.In view of this, the study was undertaken to generate baseline information on the levels of aflatoxin B_1 in compound cattle feed and raw materials for all over India from different regions at different seasons with the objective to guide compound feed manufacturers to formulate feed based on the intensity of aflatoxins in various feed ingredients, regional prevalence, incidence of occurrence during different season and revise feed formulation accordingly.

MATERIALS AND METHODS

In order to identify regional occurrence of aflatoxin, India was demarcated in this study into four zones viz. north (726133 km^2), south (636236km^2), east (425432km^2) and west (508042 km^2). A total of 2676 samples comprising of commercially available composite cattle feed samples and raw materials like maize (*Zea maize*), sorghum (*Sorghum vulgaris*) mechanical pressed groundnut oil cake (*Arachis hypogeia*), cotton seed oil cake (*Gossypium arboreum*), mustard oil cake (*Brassicanigra)*, soyabean meal (*Glycine max*), rapeseed meal (*Brassica napus),* solvent extracted cakes of groundnut (*Arachis hypogeia*) andcotton seed (*Gossypium arboreum*), rice bran (*Avena sativa*), deoiledrice bran(*Avena sativa*) and wheat bran (*Triticumaestivum)* were collected from four zones of India during a period of five years. All the samples were collected from different cooperative cattle feed plants and farmers from four zones of India. The east zone contains the states of Odisha, West Bengal, Bihar, Jharkhand, Chattisgarh, Assam, Arunachal Pradesh, Nagaland, Tripura, Mizoram, and Meghalya. The geographical area of east zone is 425432 KM sq.The west zone includes Gujarat, Maharastra, Daman and Diu, Dadra and Nagar Haveli with geographical area to the extent of 508042 KM sq. The North zone includes the states of Jammu Kashmir, Delhi, Punjab, Haryana, Rajasthan, Chandigarh, Himachal Pradesh, Utterpradesh, Uttharkhand and Madhyapradesh with geographical area to the extent of 726133 KM Sq. The south zone includes the states of Tamil Nadu, Andhra Pradesh, Telangana, Karnataka, Kerala, Andaman and Nicobar and Lakshadeep with geographical area to the extent of 636236 KM sq. The samples were collected from above regions throughout the year for the analysis of aflatoxins.

In order to identify seasonal variation, the periods were separated into two, namely non-monsoon and monsoon seasons according to local meteorological parameters. The samples were finely ground in a willy mill and one kg material was mixed thoroughly for homogeneity. Portion of 100g sub samples were drawn for aflatoxin analysis by HPLC and ELISA methods. The samples were screened by ELISA method for aflatoxin B_1 (Patey *et al.*,1992). Acetonitrile was used to extract toxins from the ground-up sample. In ELISA method, the limited level (Max. 0.4 ppb) of aflatoxin B_1could be tested.

The samples that were beyond the deduction level of ELISA method (50ppb) were analysed by HPLC. The feed sample was extracted and purified as per AOAC (2012).The HPLC conditions were used to separate the aflatoxin B_1. Mobile phase: Actonitrile: Water (65: 35) with 0.5ml flow rate, ambient temperature for column (C18 ODS, 125 x 4mm), Florescence detector: Ex. 365nm and Em. 430nm. The sensitivity for aflatoxin detection by HPLC was 20picogram.The samples were quantified in the ranges of 0-20ppb (IS 2052:2009) recommended level) and considered as "safe", while aflatoxin $B_1$from 21 to 100ppb was considered as "High". Since production and health of dairy herds are affected at dietary aflatoxin levels above 100ppb which is considerably higher than the amount that produces illegal milk residues (Patterson and Anderson 1982 and Masri *et al.*, 1969), concentration of aflatoxin B_1 more than 100ppb was considered as 'Alarmingly high'.

RESULTS AND DISCUSSIONS

Average aflatoxin B_1 (ppb) content in the composite cattle feed and raw materials are presented in Table 1. The average level of aflatoxin $B_1$with Mean± SE in 482 samples of composite cattle feed tested across India was 46.40 ± 2.68 with 68.5per cent of samples within permissible level(<20 ppb) and 2.3per cent(11 numbers) at alarming high (>100ppb) level. Several authors have reported aflatoxin B_1 levels varying from 100 to 1000ppb. (Reddy *et al.*, 2000; Lanyasunya *et al*., 2005; Charoenpornsook and Kavisarasai, 2006; Martins *et al.*, 2007). Similar incidence of contamination of alflatoxin B_1 (33per cent) in composite feed in India was recorded Kotinagu *et al.* (2015). The global prevalence of aflatoxin positive samples and the mean concentration were reported to be 33per cent and 21 ppb, respectively (Andrea *et al*., 2017). Incidence of multiple aflatoxicosis outbreaks have been reported worldwide, in Kenya (Kangíethe and Langía, 2009), in Thailand (Charoenpornsook and Kavisarasai., 2006).Countries in Europe including Romania, Serbia, and Croatia also reported the aflatoxin contamination of milk nationwide, Abbas *et al.* (2010). The carry-over level of aflatoxin B_1 to aflatoxin M_1 varies from 0.1per cent to 5per cent. (Lynch, 1971; Fremy *et al.*, 1988; Hans *et al.*, 2000; Garg *et al.*, 2004).A thumb rule is that milk aflatoxin concentrations replace equal about 1.7per cent of the aflatoxin concentration in the total ration dry matter (Whitlow and Hagler, 2005). Thus, cows consuming diets containing 46 ppb aflatoxin will produce milk containing aflatoxin residues of about 0.78 ppb which is 15 times higher than 0.05 ppb which is the safe limit for aflatoxin M_1 in milk (Codex, 2001).Five energy source, eight protein supplements and three by products that are commonly included as feed ingredients in composite cattle feed were also tested. Among energy source, maize had the highest (7.8per cent of 193 samples tested) prevalence of alarming level (>100ppb)and 41.5per cent in the range of 21 to 100 ppb of aflatoxin B_1. Only 50.8per cent(98 samples)were within permissible level (<20ppb). The mean ± SE level of aflatoxin B_1 in 193 samples of maize was found to be 61.75 ± 16.95 and was the second most highly significant ($P<0.01$) infested feed ingredient that is widely used in cattle feed. However, the level of aflatoxin B_1 in maize was highly variable as reflected

by high standard error associated to mean value. Next in the order of prevalence of aflatoxin B_1 among energy source was broken rice. Though 80.4per cent of broken rice (194 samples) had aflatoxin B_1 within permissible level, the mean ± SE of 36.60 ± 8.59 was significantly (P<0.01) higher than the rest of energy sources tested. Further broken rice had alarming level of aflatoxin B_1 in 2.0per cent of samples tested. The wheat, sorghum and Pearl millets with 85.8, 96.0and 100per cent of 155, 125 and 121 respective samples tested were relatively safe with aflatoxin B_1 level less than 20ppb. Higher incidence of aflatoxin B_1 in maize at higher concentration recorded in this study concurs with the results (1000ppb) published by Sinha (1987).

Table 1. Prevalence of aflatoxin B_1 in composit cattle feed and feed ingredients in India

Sr. No	Ingredients	No. of Samples	Aflatoxin B_1 (ppb) mean with SE*	Aflatoxin B_1 Concentraztion (ppb) and its percentage of Samples tested					
				ND-20	% Safe	21-100	% High	>100	% Alarming
A	**Composite Feed**								
1	Composite Cattle feed	482	46.40 ± 2.68^{c}	330	68.5	141	29.3	11	2.3
B	**Energy source**								
1	Maize	193	61.75 ± 16.95^{b}	98	50.8	80	41.5	15	7.8
2	Wheat	155	28.43 ± 9.85^{e}	133	85.8	22	14.2		0.0
3	Sorghum	125	8.66 ± 0.67^{g}	120	96.0	5	4.0		0.0
4	Broken rice	194	36.60 ± 8.59^{d}	156	80.4	36	18.6	2	1.0
5	Pearl millet	121	9.55 ± 1.45^{g}	121	100.0	0	0.0		0.0
C	**Protein supplement**								
1	Groundnut Cake	250	71.16 ± 9.17^{a}	118	47.2	110	44.0	22	8.8
2	Cotton Seed Cake	151	46.29 ± 7.66^{c}	128	84.8	23	15.2		0
3	Mustard cake	145	13.42 ± 9.68^{fg}	125	86.2	20	13.8		0
4	Rape seed meal	134	17.44 ± 2.55^{f}	116	86.6	18	13.4		0
5	Soyabean meal	152	11.19 ± 7.01^{g}	134	88.2	18	11.8		0
6	Sunflower meal	132	16.01 ± 9.76^{f}	124	93.9	8	6.1		0
7	Guar meal	117	8.43 ± 3.16^{g}	117	100.0	0	0.0		0
8	Safflower meal	112	9.57 ± 8.89^{g}	112	100.0	0	0.0		0
D	**By Products**								
1	Deoiled rice bran	81	10.70 ± 6.91^{g}	77	95.1	4	4.9		0
2	Wheat bran	132	12.71 ± 9.35^{fg}	128	97.0	4	3.0		0

*Means bearing different alphabets in a column (p<0.01) highly Significantly

The highest (8.8per cent of 250 samples) incidence of alarming level (>100ppb) aflatoxin $B_1$was recorded in groundnut cake. Only 47.2per cent of Groundnut cake (118 samples) were within permissible level of <20ppb of aflatoxin B_1. The mean level of aflatoxin B_1 with SE (71.16 ± 9.17) was significantly (P<0.01) highest in Groundnut cake across all samples tested. Similar observation of 41 to 51per cent of maize samples being infested in Kenya was reported by Johnni *et al.* (2011). Sharma *et al.* (1994) reported highest level of 2000ppb in groundnut cake and Bhat *et al.* (1996) reported maximum level of contamination of 833ppb in the state of Gujarat.

The cotton seedcake was the next highest source of aflatoxin B_1among protein supplements with 84.8per cent of 151 samples tested having <20ppb and mean level of aflatoxin B_1 with SE of 46.29 ± 7.66. The mean ± SE of cotton seed cake was significantly (P<0.01) higher than the rest of protein supplements tested. Other protein supplements like mustard cake, rape seed meal, soyabean meal, sunflower meal, gaur meal and safflower meal were relatively safe with aflatoxin B_1 at less than 20ppb in 86.2, 86.6, 88.2, 93.0, 100 and 100per cent respectively of 145, 134, 152, 132, 117 and 112 samples tested.

None of the by-products namely, deoiled rice bran and wheat bran had aflatoxin B_1 at alarming level and majority (95.1, 97.0per cent of 81 and 132respectively of the samples tested) were safe with aflatoxin B_1 within permissible level. The mean and SE values were significantly (P<0.01) lower with10.70 ± 6.91 and 12.71 ± 9.35, respectively.

Phitsanu and Yoshiko (2017) observed that 30.5per cent of peanuts, corn and rice showed an aflatoxin B_1 contamination ranging from 0.01-626ppb that are very similar to the observations made in this study. Maize, groundnuts and rice represent the most common sources of food supply contamination worldwide, as these crops are preferred by *Aspergillus* for colonization pre-harvest, while also being susceptible to contamination due to improper drying and storage conditions post-harvest (CAST, 2003).

The incidence of aflatoxin B_1 in composite cattle feed and raw materials tested from four zones in India are presented in table 2. The table classifies data as "sample profile" which denotes the proportion of aflatoxins B_1 concentration within each zone. This data was reclassified to represent zone wise distribution. In order to elicit the effect of environment, the data was divided according to period of origin (monsoon and non-monsoon) and presented zone-wise as well as across zones to reveal overall picture in Table 3.Thecomposite cattle feed in west zone recorded lowest sample profile (60per cent) and zone-wise distribution percentage (21.69) of samples within the permissible level (<20ppb) of aflatoxin B_1,while in other three zones, sample profile ranged from 68.46 to 72.81 and zone-wise distribution ranged from 25.00 to 26,81per cent. This is indicative of higher incidence of aflatoxin B_1 in west zone than other three zones. Data rearranged across zone to elicit the effect of monsoon indicate that relatively lower proportion (67.86per cent) of composite cattle feed were within safety limit (<20ppb) during monsoon compared to 74.42per cent in non-monsoon period. Period-wise assessment in each zone on the percentage of samples falling within safety limit revealed that only 31.43per cent of the composite cattle feed samples analysed were within safety limit in west zone during monsoon, while it ranged from 56.25 to 67.86 in other zones. During non-monsoon period, these values ranged from 71.46 to 75.00 per cent in four zones. Overall data reveals that monsoon favours the development of aflatoxin B_1 more during monsoon in west zone than other zones. This observation is further reinforced by the data pertaining to incidence of aflatoxin B_1concentrations between 21 to 100ppb wherein the

percentage of samples at west zone during monsoon was 65.71 as against the range of 28.13to 39.29 in other three zones. In contrast, it was observed that incidence of aflatoxin B_1 at concentration beyond 100ppb was relatively higher in eastern zone both during monsoon and non-monsoon period than the west, north and south zone. Thus, seasonal changes in ingredient composition of composite cattle feed or conducive environment during monsoon alone cannot be attributed to incidence of aflatoxin B_1 as no uniform pattern were noticed. However, contaminations with *Aspergillus flavus* as well as the presence of aflatoxin B_1 have been linked with the onset and intensity of the monsoon and subsequent storage conditions of the feed (Mehan and McDonald, 1983).The lower incidence of aflatoxin B_1 at concentration below100ppb and higher incidence at beyond 100ppb in east zone indicates that at some pockets the composite cattle feed were stored for longer duration leading to skewing up higher incidence at beyond 100ppb.In concurrence to the observation made in this study, higher incidence of aflatoxin B_1upto 833ppb was observed in the state of Gujarat (Bhat *et al.*, 1996), which falls under western zone.

Table 2. Incidence of aflatoxin B_1 (Mean and respective percentage of total) in composite cattle feed and raw materials tested from four Zones in India.

Sr. No	Ingredients	Level of aflatoxin B1 (ppb)	No of samples / percentage from different sources at four zones												
			Sample profile								Zone wise percetage of sample				
			East		West		North		South						
	Seasons		Number of sample	Profile Percentage	Number of sample	Profile Percentage	Number of sample	Profile Percentage	Number of sample	Profile Percentage	East	West	North	South	Total
A	Composite cattle feed	ND -20	89	68.46	72	60.00	83	72.81	88	72.13	26.81	21.69	25.00	26.51	100.00
		21-100	32	24.62	46	38.33	31	27.19	34	27.87	22.38	32.17	21.68	23.78	100.00
		>100	9	6.92	2	1.67	0	0.00	0	0.00	81.82	18.18	0.00	0.00	100.00
		Total	**130**	**100.00**	**120**	**100.00**	**114**	**100.00**	**122**	**100.00**					
B	Grains														
1	Maize	ND -20	25	48.08	26	52.00	26	46.43	21	38.89	25.51	26.53	26.53	21.43	100.00
		21-100	23	44.23	21	42.00	27	48.21	28	51.85	23.23	21.21	27.27	28.28	100.00
		>100	4	7.69	3	6.00	3	5.36	5	9.26	26.67	20.00	20.00	33.33	100.00
		Total	**52**	**100.00**	**50**	**100.00**	**56**	**100.00**	**54**	**100.00**	**75.41**	**67.74**	**73.80**	**83.04**	
2	Wheat	ND -20	25	78.13	34	89.47	21	91.30	18	66.67	25.51	34.69	21.43	18.37	100.00
		21-100	7	21.88	4	10.53	2	8.70	9	33.33	31.82	18.18	9.09	40.91	100.00
		Total	**32**	**100.00**	**38**	**100.00**	**23**	**100.00**	**27**	**100.00**					
3	Sorghum	ND -20	31	100.00	34	97.14	27	96.43	29	93.55	25.62	28.10	22.31	23.97	100.00
		21-100	0	0.00	1	2.86	1	3.57	2	6.45	0.00	25.00	25.00	50.00	100.00
		Total	**31**	**100.00**	**35**	**100.00**	**28**	**100.00**	**31**	**100.00**					
4	Broken rice	ND -20	37	82.22	49	96.08	37	77.08	33	66.00	23.72	31.41	23.72	21.15	100.00
		21-100	8	17.78	2	3.92	11	22.92	15	30.00	22.22	5.56	30.56	41.67	100.00
		>100	0	0.00	0	0.00	0	0.00	2	4.00	0.00	0.00	0.00	100.00	100.00
		Total	**45**	**100.00**	**51**	**100.00**	**48**	**100.00**	**50**	**100.00**					
3	Pearl millet	ND -20	31	100.00	31	100.00	22	100.00	37	100.00	25.62	25.62	18.18	30.58	100.00
		Total	**31**	**100.00**	**31**	**100.00**	**22**	**100.00**	**37**	**100.00**					
C	Protein meals/Oil Cakes														
1	Groundnut cake	ND -20	28	46.67	25	38.46	28	45.90	37	57.81	23.73	21.19	23.73	31.36	100.00
		21-100	27	45.00	30	46.15	28	45.90	25	39.06	24.55	27.27	25.45	22.73	100.00
		>100	5	8.33	10	15.38	5	8.20	2	3.13	22.73	45.45	22.73	9.09	100.00
		Total	**60**	**100.00**	**65**	**100.00**	**61**	**100.00**	**64**	**100.00**					
2	Cottonseed cake	ND -20	22	61.11	26	63.41	22	61.11	24	63.16	23.40	27.66	23.40	25.53	100.00
		21-100	14	38.89	15	36.59	14	38.89	14	36.84	24.56	26.32	24.56	24.56	100.00
		Total	**36**	**100**	**41**	**100**	**36**	**100**	**38**	**100**					

3	Mustard cake	ND -20	36	85.71	35	85.37	34	87.18	20	86.96	28.80	28.00	27.20	16.00	100.00
		21-100	6	14.29	6	14.63	5	12.82	3	13.04	30.00	30.00	25.00	15.00	100.00
		Total	**42**	**100**	**41**	**100**	**39**	**100**	**23**	**100**					
4	Rape seed meal	ND -20	32	86.49	34	87.18	31	86.11	19	86.36	27.59	29.31	26.72	16.38	100.00
		21-100	5	13.51	5	12.82	5	13.89	3	13.64	27.78	27.78	27.78	16.67	100.00
		Total	**37**	**100**	**39**	**100**	**36**	**100**	**22**	**100**					
5	Soyabean meal (extracted)	ND -20	41	89.13	30	85.71	35	89.74	28	87.50	30.60	22.39	26.12	20.90	100.00
		21-100	5	10.87	5	14.29	4	10.26	4	12.50	27.78	27.78	22.22	22.22	100.00
		Total	**46**	**100**	**35**	**100**	**39**	**100**	**32**	**100**					
6	Sunflower meal	ND -20	28	93.33	31	93.94	31	93.94	34	94.44	22.58	25.00	25.00	27.42	100.00
		21-100	2	6.67	2	6.06	2	6.06	2	5.56	25.00	25.00	25.00	25.00	100.00
		Total	**30**	**100**	**33**	**100**	**33**	**100**	**36**	**100.00**					
7	Guar meal	ND -20	29	100	31	100.00	31	100.00	26	100.00	24.79	26.50	26.50	22.22	100.00
		Total	**29**	**100**	**31**	**100**	**31**	**100**	**26**	**100.00**					
8	Safflower meal	ND -20	28	100	27	100.00	29	100.00	28	100.00	25.00	24.11	25.89	25.00	100.00
		Total	**28**	**100**	**27**	**100**	**29**	**100**	**28**	**100.00**					
D	**Brans**														
1	Deoiled Rice bran	ND -20	17	94.44	19	95.00	18	94.74	23	95.83	22.08	24.68	23.38	29.87	100.00
		20-100	1	5.56	1	5.00	1	5.26	1	4.17	25.00	25.00	25.00	25.00	
		Total	**18**	**100**	**20**	**100**	**19**	**100**	**24**	**100.00**					
2	Wheat bran	ND -20	35	97.22	36	97.30	28	96.55	29	96.67	27.34	28.13	21.88	22.66	100.00
		20-50	1	2.78	1	2.70	1	3.45	1	3.33	25.00	25.00	25.00	25.00	
		Total	**36**	**100**	**37**	**100**	**29**	**100**	**30**	**100.00**					

The maize sample profile data of south zone suggest that prevalence of aflatoxin B_1below 20ppb was 38.89per cent of samples received and was relatively lower than other three zones. Zone wise segregation of samples also revealed only 21.43per cent of samples from south zone were within safe limit of aflatoxin B_1. Incidentally, maize is not largely consumed by human in south zone. Maize is widely cultivated and consumed by human in west and north zones. Period-wise occurrence of aflatoxin B_1in maize at concentration above 20ppb across four zones indicated that samples tested during monsoon were more prone to aflatoxin B_1than those tested during non-monsoon period even though non-monsoon lasts for ten months as against two months of monsoon period. Similarly, the sample profile, zone-wise distribution of samples, period-wise incidence in each zone and across all zones of other cereals like wheat, sorghum and broken rice were all found to be higher during monsoon in south zone than other three zones. All the pearl millet samples analysed were within safe limit of aflatoxin B_1. Thus, it is evident that prevalence of aflatoxin B_1in cereals was found to be higher during monsoon in south zone than other three zones.

The incidence of aflatoxin B_1 in groundnut cake was found to be highest among all samples tested. In contrast to cereals, south zone samples of groundnut cake were found to have lower incidence of aflatoxin B_1as indicated by sample profile data and zone-wise segregation of sample results. The west zone recorded lowest sample profile (38.46per cent) and the zone profile (21.19per cent) of samples within the permissible level of (<20ppb) aflatoxin B_1, while in other three zones sample profile ranged from 45.90per cent to 57.81per cent and zone-wise distribution ranged from 23.73 to 31.36per cent. In west

Table 3. Incidence of aflatoxin B_1 (Mean and respective percentage of total) in composite cattle feed and raw materials tested at two seasons from four Zones in India.

Sr. No	Ingredients	Level of aflatoxin B1 (ppb)	No of samples / percentage from different sources at four zones in different periods																			
			Sample profile - Period wise in each zone																Period wise across zone			
			East				West				North				South							
	Seasons		Non monsoon	(% during non monsoon within zone)	Monsoon	(% during non monsoon within zone)	Non monsoon	(% during non monsoon within zone)	Monsoon	(% during monsoon within zone)	Non monsoon	(% during non monsoon within zone)	Monsoon	(% during monsoon within zone)	Non monsoon	(% during non monsoon within zone)	Monsoon	(% during monsoon within zone)	Non monsoon	(% during non monsoon within zone)	Monsoon	(% during monsoon within zone)
A	Composite cattle feed	ND -20	71	74.0	18	56.3	61	71.8	11	31.4	64	74.4	19	67.9	69	75.0	17	60.7	265	73.8	65	52.8
		21-100	21	21.9	9	28.1	23	27.1	23	65.7	22	25.6	9	32.1	23	25.0	11	39.3	89	24.8	52	42.3
		>100	4	4.2	5	15.6	1	1.2	1	2.9	0	0.0	0	0.0	0	0.0	0	0.0	5	1.4	6	4.9
		Total	**96**	**100.0**	**32**	**100.0**	**85**	**100.0**	**35**	**100.0**	**86**	**100.0**	**28**	**100.0**	**92**	**100.0**	**28**	**100.0**	**359**	**100.0**	**123**	**100.0**
B	**Grains**																					
1	Maize	ND -20	19	51.4	6	40.0	23	59.0	3	27.3	24	77.4	2	33.3	18	51.4	3	15.8	84	59.2	14	27.5
		21-100	16	43.2	7	46.7	15	38.5	6	54.5	5	16.1	3	50.0	15	42.9	13	68.4	51	35.9	29	56.9
		>100	2	5.4	2	13.3	1	2.6	2	18.2	2	6.5	1	16.7	2	5.7	3	15.8	7	4.9	8	15.7
		Total	**37**	**100.0**	**15**	**100.0**	**39**	**100.0**	**11**	**100.0**	**31**	**100.0**	**6**	**100.0**	**35**	**100.0**	**19**	**100.0**	**142**	**100.0**	**51**	**100.0**
2	Wheat	ND -20	15	83.3	20	83.3	48	96.0	11	84.6	10	90.9	11	91.7	15	83.3	3	33.3	88	90.7	45	77.6
		21-100	3	16.7	4	16.7	2	4.0	2	15.4	1	9.1	1	8.3	3	16.7	6	66.7	9	9.3	13	22.4
		Total	**18**	**100.0**	**24**	**100.0**	**50**	**100.0**	**13**	**100.0**	**11**	**100.0**	**12**	**100.0**	**18**	**100.0**	**9**	**100.0**	**97**	**100.0**	**58**	**100.0**
3	Sorghum	ND -20	22	100.0	9	100.0	16	100.0	18	94.7	21	100.0	6	85.7	12	92.3	17	94.4	71	98.6	50	94.3
		21-100	0	0.0	0	0.0	0	0.0	1	5.3	0	0.0	1	14.3	1	7.7	1	5.6	1	1.4	3	5.7
		Total	**22**	**100.0**	**9**	**100.0**	**16**	**100.0**	**19**	**100.0**	**21**	**100.0**	**7**	**100.0**	**13**	**100.0**	**18**	**100.0**	**72**	**100.0**	**53**	**100.0**
4	Broken rice	ND -20	23	82.1	14	82.4	31	96.9	18	94.7	24	82.8	13	68.4	21	70.0	12	60.0	99	83.2	57	76.0
		21-100	5	17.9	3	17.6	1	3.1	1	5.3	5	17.2	6	31.6	8	26.7	7	35.0	19	16.0	17	22.7
		>100	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	3.3	1	5.0	1	0.8	1	1.3
		Total	**28**	**100.0**	**17**	**100.0**	**32**	**100.0**	**19**	**100.0**	**29**	**100.0**	**19**	**100.0**	**30**	**100.0**	**20**	**100.0**	**119**	**100.0**	**75**	**100.0**
3	Pearl millet	ND -20	15	100.0	16	100.0	11	100.0	20	100.0	**4**	100.0	18	100.0	**14**	100.0	23	100.0	44	100.0	77	100.0
		Total	**15**	**100.0**	**16**	**100.0**	**11**	**100.0**	**20**	**100.0**	**4**	**100.0**	**18**	**100.0**	**14**	**100.0**	**23**	**100.0**	**44**	**100.0**	**77**	**100.0**
C	**Protein meals/Oil Cakes**																					
1	Groundnut cake	ND -20	14	48.3	14	45.2	13	44.8	12	33.3	14	51.9	14	41.2	19	59.4	18	56.3	60	51.3	58	43.6
		21-100	14	48.3	13	41.9	12	41.4	18	50.0	11	40.7	17	50.0	13	40.6	12	37.5	50	42.7	60	45.1
		>100	1	3.4	4	12.9	4	13.8	6	16.7	2	7.4	3	8.8	0	0.0	2	6.3	7	6.0	15	11.3
		Total	**29**	**100.0**	**31**	**100.0**	**29**	**100.0**	**36**	**100.0**	**27**	**100.0**	**34**	**100.0**	**32**	**100.0**	**32**	**100.0**	**117**	**100.0**	**133**	**100.0**

2	Cottonseed cake	ND -20	11	57.9	11	64.7	15	71.4	11	55.0	13	68.4	9	52.9	12	70.6	12	57.1	51	67.1	43	57.3
		21-100	8	42.1	6	35.3	6	28.6	9	45.0	**6**	**31.6**	8	47.1	5	29.4	9	42.9	25	32.9	32	42.7
		Total	**19**	**100.0**	**17**	**100.0**	**21**	**100.0**	**20**	**100.0**	**19**	**100.0**	**17**	**100.0**	**17**	**100.0**	**21**	**100.0**	**76**	**100.0**	**75**	**100.0**
3	Mustard cake	ND -20	18	81.8	18	90.0	14	87.5	21	84.0	22	95.7	12	75.0	4	80.0	16	88.9	58	87.9	67	84.8
		21-100	4	18.2	2	10.0	2	12.5	4	16.0	1	4.3	4	25.0	1	20.0	2	11.1	8	12.1	12	15.2
		Total	**22**	**100.0**	**20**	**100.0**	**16**	**100.0**	**25**	**100.0**	**23**	**100.0**	**16**	**100.0**	**5**	**100.0**	**18**	**100.0**	**66**	**100.0**	**79**	**100.0**
4	Rape seed meal	ND -20	22	91.7	10	76.9	23	92.0	11	78.6	18	90.0	13	81.3	10	100.0	9	75.0	73	92.4	43	78.2
		21-100	2	8.3	3	23.1	2	8.0	3	25.0	2	10.0	3	18.8	0	0.0	3	25.0	6	7.6	12	21.8
		Total	**24**	**100.0**	**13**	**100.0**	**25**	**100.0**	**14**	**103.6**	**20**	**100.0**	**16**	**100.0**	**10**	**100.0**	**12**	**100.0**	**79**	**100.0**	**55**	**100.0**
5	Soyabean meal (extracted)	ND -20	23	92.0	18	85.7	18	90.0	12	80.0	19	90.5	16	88.9	19	90.5	9	81.8	79	90.8	55	84.6
		21-100	2	8.0	3	14.3	2	10.0	3	20.0	2	9.5	2	11.1	2	9.5	**2**	18.2	8	9.2	10	15.4
		Total	**25**	**100.0**	**21**	**100.0**	**20**	**100.0**	**15**	**100.0**	**21**	**100.0**	**18**	**100.0**	**21**	**100.0**	**11**	**100.0**	**87**	**100.0**	**65**	**100.0**
6	Sunflower meal	ND -20	18	100.0	10	83.3	17	100.0	14	87.5	16	94.1	15	93.8	18	100.0	16	88.9	69	98.6	55	88.7
		21-100	0	0.0	2	16.7	0	0.0	2	12.5	1	5.9	1	6.3	0	0.0	2	11.1	1	1.4	7	11.3
		Total	**18**	**100.0**	**12**	**100.0**	**17**	**100.0**	**16**	**100.0**	**17**	**100.0**	**16**	**100.0**	**18**	**100.0**	**18**	**100.0**	**70**	**100.0**	**62**	**100.0**
7	Guar meal	ND -20	14	100.0	15	100.0	13	100.0	18	100.0	15	100.0	16	100.0	0	0.0	0	**0.0**	55	100.0	62	100.0
		Total	**14**	**100.0**	**15**	**100.0**	**13**	**100.0**	**18**	**100.0**	**15**	**100.0**	**16**	**100.0**	**0**	**0.0**	**0**	**0.0**	**55**	**100.0**	**62**	**100.0**
8	Safflower meal	ND -20	**13**	**100.0**	15	100.0	14	100.0	**13**	100.0	14	100.0	**15**	100.0	13	100.0	15	100.0	54	100.0	58	100.0
		Total	**13**	**100.0**	**15**	**100.0**	**14**	**100.0**	**13**	**100.0**	**14**	**100.0**	**15**	**100.0**	**13**	**100.0**	**15**	**100.0**	**54**	**100.0**	**58**	**100.0**
D	**Brans**																							
1	Deoiled Rice bran	ND -20	11	100.0	6	85.7	12	100.0	7	87.5	10	100.0	8	88.9	11	100.0	12	92.3	44	100.0	33	89.2
		20-100	0	0.0	1	14.3	0	0.0	1	12.5	0	0.0	1	11.1	0	0.0	1	7.7	0	0.0	4	10.8
		Total	**11**	**100.0**	**7**	**100.0**	**12**	**100.0**	**8**	**100.0**	**10**	**100.0**	**9**	**100.0**	**11**	**100.0**	**13**	**100.0**	**44**	**100.0**	**37**	**100.0**
2	Wheat bran	ND -20	17	100.0	18	94.7	12	100.0	24	96.0	12	100.0	16	94.1	15	100.0	14	93.3	56	100.0	72	94.7
		20-50	0	0.0	1	5.3	0	0.0	1	4.0	0	0.0	1	5.9	0	0.0	1	6.7	0	0.0	4	5.3
		Total	**17**	**100.0**	**19**	**100.0**	**12**	**100.0**	**25**	**100.0**	**12**	**100.0**	**17**	**100.0**	**15**	**100.0**	**15**	**100.0**	**56**	**100.0**	**76**	**100.0**

zone, highest sample profile (15.18per cent) and zone-wise distribution of 45.45per cent was recorded in the samples of groundnut cake having aflatoxin B_1 at alarmingly high level of more than 100 ppb. Though the overall incidence of aflatoxin B_1across zone in groundnut cake was higher during monsoon period, the south zone recorded lower incidence during monsoon than non-monsoon period.

Data on cottonseed cake sample profile and zone-wise segregation of samples revealed that prevalence of aflatoxin B_1 was uniformly infested across zone and incidence was higher during monsoon. Though mustard seed sample profile also indicated uniform distribution within respective zone, zone-wise segregation of samples revealed that prevalence of aflatoxin B_1 was relatively more in east and west zone with south being the least. Similarly, Rapeseed meal also revealed uniform sample profile with least prevalence of aflatoxin B_1 in south zone. Soyabean meal and sunflower meal were uniformly infested both on sample profile as well as on zone-wise assessment similar to cotton seed cake. All the samples of guar meal and safflower meal tested were within the safe limit of aflatoxin B_1.The incidence of aflatoxin B_1 was higher during monsoon in mustard seed, rapeseed meal, soyabean meal and sunflower meal. The overall prevalence of aflatoxin B_1 was least in protein supplements and highest in cereals in south zone.

Samples of deoiled rice bran and wheat bran revealed uniform aflatoxin B_1 profile as well as similar zone-wise distribution and the incidence of aflatoxin B_1 occurred only during monsoon.

CONCLUSIONS

This study was conducted to generate the baseline information on the level of aflatoxin B_1 content in compound cattle feed and all raw materials from different regions of country. Aflatoxin B_1 was found to be at an alarming level in most commonly used feed ingredients (> 100 ppm: groundnut cake 8.8per cent, maize7.8per cent) as well in composite cattle feed (2.3per cent) among 2676samples tested. Only 68.5 per cent of 482 composite cattle feed samples analysed were safe with aflatoxin B_1 below 20ppb. Higher incidence of aflatoxin B_1 was recorded in west zone than east, north and south zones during monsoon period. Thus, seasonal changes in ingredient composition of composite cattle feed or conducive environment during monsoon alone cannot be attributed to incidence of aflatoxin B_1 as no uniform pattern were noticed. Among energy source, maize had the highest prevalence of alarming level (>100ppb) and it was observed that the incidence of aflatoxin $B_1$was more in maize during monsoon in south zone. In west zone, highest sample profile (15.18per cent) of groundnut cake having aflatoxin B_1 at alarmingly high level of more than 100ppb.Overall data reveals that monsoon favours development of aflatoxin $B_1$is more during monsoon. This study reveals that no single factor can be attributed to the high incidence of aflatoxin B1, though the major ingredients viz., maize and groundnut cake were found to be highly prone to aflatoxin B_1. However, it is strongly recommended not to source maize from south zone during monsoon period and groundnut from west zone during monsoon period. During monsoon period, ration formulation with

sorghum and peral millet as energy source and sunflower meal, guar meal, safflower meal for protein source can be considered in addition to by products like deoiledrice bran and wheat bran. This study recommends stringent action to control aflatoxin B_1 to ensure not only animal health but also to prevent its cascading effect on human health.

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Logit analysis for the entry of farmers into contract Japanese quail farming

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ABSTRACT

The present study was undertaken to analyze the factors determining the participation of farmers in contract Japanese quail farming. The primary data for the study was collected through a pre-tested interview schedule. The determinant factors for farmers participation in contract Japanese quail farming were assessed by the logit model and the results of the analysis revealed that the farm size and profitability were found to be the positive determinant factors and investment was found to be a negative determinant factor for the participation of farmers in contract Japanese quail farming.

Key Words: Contract Japanese quail farming, Logit model, Farm size, Profitability, Investment

INTRODUCTION

Poultry is one of the important components of Animal Husbandry, which provides additional means of employment opportunities to a large number of people with the new and innovative approaches practiced. One of the innovative approaches getting popular now is an institutional arrangement that enables farmers to access markets called as 'Contract Farming'. Contract farming is a viable alternative farming model in India, which can provide assured and reliable input services to the farmers and desired farm produce to the contracting firms. The concept of contract farming promises i) to provide a proper linkage between the farm and market ii) promote high degree of competition at the supply and market end and iii) minimize intermediaries in order to increase farmer's income.

The alternate poultry production in India is gaining momentum and attention from the farmers, entrepreneurs, professionals and researchers. Among different types of contract poultry farming, contract Japanese quail farming is relatively a new venture in Tamil Nadu. As the Indian economy grows, there will be an increase in the number of people with high disposable income and consciousness about quality and health who will demand food products of certain specifications. Japanese quail is an efficient biological machine for converting feed into animal protein of high biological value and hence is one of the cheapest

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sources of animal protein for human diet. Japanese quail has unique qualities of hardiness and adaptability to diversified agro-climatic conditions. The population trend appears to be stable, and hence the species does not approach the thresholds for vulnerable under the population trend criterion (>30% decline over ten years or three generations). For these reasons, the species is evaluated as least concern. (Arya *et al.*, 2018)

Japanese quail production requires less investment and gives quick returns, higher profits and hence can be adopted by rural masses quickly. A very important point is that so far the benefits of Japanese quail farming have not fully reached the rural masses.

The inherent lacunae associated with contract Japanese quail farming are yet to be documented. No systematic study was carried out to find the determinant factors of participation of farmers in contract Japanese quail farming. Keeping the factors in mind, the present study was designed with the objective of finding the factors determining participation of farmers in contract Japanese quail farming.

MATERIALS AND METHODS

For the present study, the western zone of Tamil Nadu was purposively selected since the districts in this zone (Erode, Tiruppur and Coimbatore) have high concentration of Japanese quail farming activities. The primary data for the present study was collected from thirty contract Japanese quail farmers with a well-designed pre-tested interview schedule.

To assess the determinant factors of participation in contract Japanese quail farming, logit model was used. In explaining a dichotomous dependent variable (Y_i), where "one" represents participation of farmers in contract Japanese quail farming and "zero" represents participation in non-contract Japanese quail farming, a logit model is used to examine the determinant factors of participation in contract Japanese quail farming because of its simplest mathematical structure. The relationship between dependent and independent variables is non-linear; a logistic function is used to estimate the association between binary, endogenous variable (Y) and the independent variables (Xs). The following mathematical form of the model was used in this study.

$$\ln\left(p_i/(1-p_i)\right) = \beta_o + \sum_{j=1}^{k} \beta_j X_{ij}$$

where, p_i is the probability of the i^{th} farm being in contract and X_k is the k^{th} explanatory variable. The dependent variable ln (p_i / (1-p_i)), in the equation is the log-odds ratio in favour of participation in contract farming (Begum and Alum, 2005)

Consideration of model variables (factors)

Factors	Definition
X_1	Age in years
X_2	Education in number of years
X_3	Gender (Male-1; Female-0)
X_4	Experience in years
X_5	Landholding in acres
X_6	Farm size of respondents
X_7	Livability in per cent
X_8	Marketing weight in Kgs
X_9	Feed efficiency of birds
X_{10}	Profitability of the farm
X_{11}	Investment in rupees

Following these arguments, the following logit model was postulated.

$$\ln (p_i / (1- p_i)) = \beta_o + \beta_1X_1 + \beta_2 X_2 + \beta_3X_3 + \beta_4X_4 + \beta_5X_5 + \beta_6X_6 + \beta_7X_7 + \beta_8X8 + \beta_9X_9 + \beta_{10}X_{10} + \beta_{11}X_{11}$$

Where,

p_i = Probability of participation in contract farming

$(1- p_i)$ = Probability of non-contract farm

β_0 = Constant term

β_i's = Coefficient

X_i = Determinant factors

RESULTS AND DISCUSSION

The results of the logit model to analyze the factors determining the participation of farmers in contract Japanese quail farming are presented in Table1. On observing the contents of the table, it could be noted that the model χ^2 was 38.368, meaning that the model is statistically significant. Among the 11 variables presumed to be the determinants of participation of farmers in contract Japanese quail farming, the factors viz., experience, farm size, profitability and initial investment were found to be statistically significant and the remaining factors were statistically non-significant ($P>0.05$). Among the significant variables, the variables, farm size ($P<0.01$), profitability ($P<0.01$) and experience

(P<0.05) were the positive determinant factors for farmers participation in contract farming. The variable initial investment (P<0.01) was found to be negatively influencing the farmers participation in contract Japanese quail farming.

Profitability is a positive determinant factor and if it is significantly more than the following costs or risks which farmers would usually encounter (both in short-run and long-run), then more farmers will decide to participate in contract Japanese quail farming. Farm size is a positive determinant factor and has a direct influence on the profit and yield of the farm. This result concurs with the result of Mishra *et al.* (2018) who found that farm size has a positive and significant impact on profits and yield. Increase in experience might improve the scientific knowledge of the farmers and could rectify their faults, if any, in running the farm and more experience made them self-reliant to take farm operation decisions and willingness to participate in contract Japanese quail farming. Increase in initial investment might increase the financial risk of the farmers and it acts as a negative determinant factor for the participation of farmers in contract Japanese quail farming. The results concur with the result of Nagaraj *et al*. (2008) who found that low initial investment was one of the factors determining farmers participation in contract farming.

The reliability of the usage of the logit model for the correct prediction of determinant factors of contract and the non-contract farming were tested by comparing the observed and predicted values. The percentage of correct prediction by the logit model was 96.70 per cent. This model was successful in predicting the contract farmers (100.00 per cent) correctly than the non-contract farmers (93.30 per cent) (Table 1).

Table 1 Determinants of participation in contract Japanese quail farming

X_i	Variables	Coefficients β_i	Wald-statistic	P-value
X_1	Age in years	0.037	0.016	0.901
X_2	Education in number of years	-0.076	0.584	0.445
X_3	Gender (Male-1; Female-0)	-0.083	1.246	0.264
X_4	Experience in years	0.230 *	3.951	0.047
X_5	Landholding in acres	0.035	0.111	0.739
X_6	Farm size of respondents	0.253 **	12.271	0.000
X_7	Livability in per cent	- 0.010	0.019	0.891
X_8	Marketing weight in Kgs	0.030	0.172	0.679
X_9	Feed efficiency of birds	0.026	0.216	0.642
X_{10}	Profitability of the farm	0.574 **	8.026	0.005
X_{11}	Investment in rupees	-0.313 **	11.253	0.001
β_0	Constant	1.840 *	4.247	0.039
Model χ^2 value = 38.368 **				
Dependant Variable : Participation in contract Japanese quail farming Yes = 1 ; No = 0				
* - P<0.05 ** - P<0.01				

Classification Table

Observed		Predicted		
		Participation in contract Japanese quail farming		Percentage correct
		Yes	**No**	
Participation in contract Japanese quail farming	**Yes**	30	0	100.00
	No	2	28	93.30
Overall Percentage				**96.70**

CONCLUSION

Among the significant factors, the variables, farm size (P<0.01), profitability (P<0.01) and experience (P<0.05) were the positive factors determining the participation of farmers in contract Japanese quail farming. The variable initial investment (P<0.01) was found to be a negative factor influencing the farmers participation in contract Japanese quail farming. Contract Japanese quail farming increases the income of participating farmers and results in better management of technology. Contract Japanese quail farming has both positive and negative aspects but benefits overweigh the negative effects which can be addressed through the involvement of institutions related to the governance of the contract farming business. However, in the present context, contract Japanese quail farming is clearly a win -win situation for both the companies and the farmers. The future of contract farming in India is quite promising due to increasing consciousness about the quality demands of the export market in the developed countries.

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Heat tolerance of crossbred female calves as indicated by Iberia heat tolerance coefficient, Benezara coefficient of adaptability and dairy search index

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ABSTRACT

A study was conducted to assess the heat tolerance of crossbred female (n=7) calves of six to twelve months of age for thirty days each in summer, monsoon and winter. Temperature humidity index (THI) was calculated at forenoon and afternoon in all the seasons and heat tolerance was carried out using Iberia heat tolerance coefficient, Benezara coefficient of adaptability and Dairy search index. The highest THI was recorded in summer afternoon while lowest was recorded in winter forenoon. There was a significant increase in THI from forenoon to afternoon in all the seasons. IHTC was within the reference value in winter forenoon while rest of the time it was below the normal. IHTC decreased significantly from forenoon to afternoon in all the seasons. BCA was above the reference value in forenoon and afternoon in all the seasons. BCA increased significantly from forenoon to afternoon in all the seasons. DSI was significantly lower in winter compared to summer. It was concluded that crossbred female calves have low heat tolerance during afternoon in all the seasons.

Key Words: Heat tolerance, season, adaptability, crossbred calves

INTRODUCTION

India is an agricultural country with livestock sector as main source of livelihood for major population of rural India. Many factors negatively impairs livestock health and production, out of all the stressors the effects caused by heat stress are much more detrimental (Rivington *et al*., 2009).

Heat stress results from a negative balance between the net amount of energy flowing from the animal to its surrounding environment and the amount of heat energy produced by the animal (Bernabucci*et al*., 2010). Heat stress results in inability of the animals to lose heat to the surroundings. Under heat stress numerous physiological responses were operated (Blackshaw and Blackshaw, 1994; Sejian*et al*., 2008; Tej*et al*., 2017) to maintain the homeostasis compromising production behind (West, 1999). Changes in physiological responses such as rectal temperature (RT), respiratory rate (RR), heart rate (HR) and pulse rate (PR) are reliable indicators for recording heat tolerance/ adaptability in cattle (Kumar *et al*., 2016). Tropical cattle

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(*Bosindicus*) are thermo-tolerant with minimum physiological changes, while temperate cattle (*Bostaurus*) are susceptible to heat stress with significant alterations in physiological variables (Hansen, 2004). However, crossbreeding has been adopted for blending the adaptability of Zebu cattle with high milking potentials of exotic breeds (Sailo*et al*., 2017) to optimize production. In real, there is a need to assess heat tolerance/ adaptability in crossbred cattle inhabiting hot and humid tropical regions of India. Knowledge on adaptability of animals to existing ambience may contribute to the adoption of procedures that improve the welfare and efficiency of production. On this background the objectives, of the present study was conducted to assess heat tolerance of crossbred female calves to pre-monsoon, monsoon and post-monsoon seasons.

MATERIALS AND METHODS

Seven crossbred female calves of six to twelve months of age were randomly selected from University Livestock Farm and Fodder Research and Development Scheme, Mannuthy, Thrissur, Kerala. The animals were kept in calf shed with appropriate facilities for feeding and watering. The animals were fed as per ICAR feeding standards (Ranjhan, 1998). The study was conducted for 30 days each in May (summer), July (monsoon) and November (winter) seasons. Same animals were used in all the three seasons. Meteorological parameters such as ambient temperature (AT, ºC) and ambient relative humidity (RH, %) were recorded at forenoon and afternoon on all days of experiment using HOBO data logger (HOBO U 12 Temp/ RH/ Light/ Ext). Temperature humidity index (THI) was calculated using the equation, THI = (0.8 X Tdb) + [(RH/100) X T db – 14.4] + 46.4 (Tdb= temperature of dry bulb, RH= relative humidity) (Mader *et al*., 2006) from daily recordings AT and RH. Physiological variables such as RT, RR and PR were measured at forenoon and afternoon in all the three seasons. RT was measured by inserting a clinical thermometer in to rectum for one minute. RR was recorded by counting the flank movements for one minute.PR was recorded by manual palpation of middle coccygeal artery pulse for one minute. Heat stress tolerance of crossbred calves was carried out using Iberia heat tolerance coefficient (IHTC= 100-10 (BT ºF–101)) (BT=body temperature, F= Fahrenheit) (Rhoad, 1942), Benezara coefficient of adaptability (BCA=BT ºC/38.33+ RR/23) (RR= respiratory rate per minute) (Benezara, 1954) and Dairy search index (DSI) = 0.5(X1/X) + (0.2 (Y1/Y) + 0.3 (Z1/Z), where X1, Y1 and Z1 are the observed RT (°C), RR and PR (per min) respectively while X, Y, and Z are normal RT, RR and PR (per min) (Thomas *et al*., 1973). The data was analyzed by comparing the means of IHTC and BCA between forenoon and afternoon in each season using unpaired t- test. Whereas for DSI, one way Analysis of variance (ANOVA) was performed for between season comparison by taking winter forenoon values (normal values) as X, Y, Z and their respective afternoon values during summer, monsoon and winter X1, Y1, Z1. The data obtained on various parameters were statistically analyzed as per the standard techniques (Snedecor and Cochran, 1994) using computerized software programme SPSS Ver. 20.

RESULTS AND DISCUSSION

Adaptability is the capacity of body's physiological system to withstand unfavourable environmental conditions without significant impairment of its normal operations (Azam*et al*., 2012). Adaptability levels vary with breed and species. Cattle from Zebu breed are thermotolerant, the consequences of exposure to heat stress for milk and meat production are less pronounced compared to temperate cattle which are susceptible to heat stress with numerous changes in neuroendocrine and physiological varaibles compromising production behind (Hansen, 2004). Thus, assessment of thermal tolerance is of paramount importance for adoption of strategies to optimize production. With this background, the current study was aimed to evaluate heat tolerance of crossbred calves by non-invasive techniques. The mean±SE of THI at forenoon and afternoon during summer, monsoon and winter seasons were presented in Table 1. The lowest ($p<0.01$) THI was recorded in winter forenoon with an overall mean value of 76.71±0.31, while highest ($p<0.01$) mean THI was recorded in summer afternoon (85.87±0.26). THI significantly ($p<0.01$) increased from forenoon to afternoon in all the three seasons. THI is a single value which accounts for the combined effect of AT and RH and is one of the most commonly used measure of heat stress in livestock (Marai and Haeeb, 2010).

A THI of above 74 was considered to cause heat stress in dairy cattle (Armstrong, 1994). In the present study a THI of above 74 was noticed during forenoon and afternoon in all the three seasons, which is indicative of heat stress in all the animals. A significant increase in THI from forenoon to afternoon in all the three seasons indicates that the quantum of heat stress was high in afternoon compared forenoon in all the three seasons. Our findings was in accordance with the reports of Tej*et al*. (2017) and Aziz *et al*. (2016) who recorded critically high THI prevailing in summer, monsoon and winter seasons and significantly higher THI in the afternoon compared to forenoon in all the seasons in Thrissur, Kerala. Similarly studies on effect of high THI of above 74 significantly altered on physiological (Vaidya*et al*., 2010; Abdelatif and Alamenn, 2012), haematological (Lateef*et al*., 2014), biochemical parameters (Tej*et al*., 2017) which are indicators of heat stress in cattle. Though, THI does not take in to account of solar radiation and wind speed, it is one of the best method to assess heat stress in cattle (Marai and Haeeb, 2010).

Table.1 Mean±SE of THI at forenoon and afternoon during summer, monsoon and winter seasons.

Season	Time	THI
Summer	00.80-00.90h	80.58^{a}±0.31
	00.13-00.14h	85.87^{b}±0.26
Monsoon	00.80-00.90h	77.15^{a} ±0.23
	00.13-00.14h	81.70^{b}±0.29
Winter	00.80-00.90h	76.71^{a}±0.31
	00.13-00.14h	82.12^{b}±0.26

Means with different superscript (a, b) in a column within a season differ significantly ($p<0.01$).

Table 2.Mean±SE values of IHCT, BCA and DSI of crossbred female calves at forenoon and afternoon during summer, monsoon and winter seasons

Season	Time	IHTC	BCA	DSI
Summer	00.80-00.90 h	95.73^{a}±0.19	4.23^{a}±0.05	1.35±0.03^{a}
	00.13-00.14 h	79.19^{b}±1.82	5.91^{b}±0.10	
Monsoon	00.80-00.90 h	98.77^{a}±1.76	4.02^{a}±0.01	1.32±0.01^{ab}
	00.13-00.14 h	78.08^{b}±1.00	5.71^{b}±0.04	
Winter	00.80-00.90 h	100.41^{a}±0.48	3.93^{a}±0.02	1.28±0.01^{b}
	00.13-00.14 h	79.41^{b}±0.41	5.64^{b}±0.03	
Reference value		100	2	1

Means with different superscript (a, b) in a column differ significantly ($p<0.05$).

The Mean±SE values of IHCT and BCA of crossbred female calves at forenoon and afternoon during summer, monsoon and winter seasons were presented in Table 2. IHTC and BCA were used to assess heat tolerance in cattle. IHTC and BCA are linear equations which take in to account of RT and RR and the resultant value obtained indicates the heat tolerance capacity or adaptability of animals to heat stress. The reference range for IHTC was found to be >100 (Rhoad, 1942) and BCA was found to be <2 (Benezara, 1954). Animals with highest IHTC and lowest BCA are considered to be highly thermotolerant. In the current study, highest IHTC (100.41±0.48) was observed during winter forenoon while lowest IHTC (78.08±1.00) was observed during monsoon afternoon. Further, IHTC decreased significantly ($p<0.05$) from forenoon to afternoon in all the seasons. BCA was found to be above the reference value at forenoon and afternoon during all the three seasons. Furthermore, BCA increased significantly ($p<0.05$) from forenoon to afternoon in all the seasons. IHTC and BCA values close to reference value was observed during winter forenoon indicating highest adaptability of calves to winter forenoon which could be due to low THI recorded at that period. However, the IHTC and BCA values were above the reference range during forenoon and afternoon in all the seasons which could be due high THI recorded in all the seasons. The present findings were in accordance with the reports of Mandal and Tyagi (2008), Das (2012), Sailo*et al*., (2017) and Kumari *et al*., (2018) where they observed a lower IHTC and BCA during winter season in cattle. A significant change in IHTC and BCA from forenoon to afternoon in all the three seasons could be due to rise in THI from forenoon to afternoon.

DSI is another commonly used heat tolerance index to assess adaptability in ruminants. An increase in DSI value from '1' indicates decrease in thermal adaptability (Kumari*et al*., 2018). ANOVA indicated significant ($p<0.05$) difference in DSI between summer and winter season with lower ($p<0.05$) value recorded in winter while higher ($p<0.05$) value recorded in summer indicating high adaptability in winter and thermal susceptibility to heat stress in summer. Similar findings were reported with high thermal adaptability in

winter and low DSI in summer in Sahiwal, Gir, Jersey cross, Holstein Friesian cross and Murrah buffalo (Kumari*et al*., 2018).

From the present study it was concluded that the animals are less thermotolerant during afternoon in all the three seasons. This could be due to failure of the calves to acclimatize with stressful diurnal variation in THI from forenoon to afternoon. High THI must have imposed significant heat stress on animals resulting in altered RT, RR and BT which could result in reduced production. Thus necessary management strategies must be employed to reduce the risk of heat stress to optimize production.

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Compliance with ethical standards

The animal studies have been approved by the appropriate ethics committee, Order No. KVASU/DAR/ACAD A(1)/11795/2014, dated 30.05.2014. Code No CB/25/38/MVM2013/PY and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

Disclosure statement

The authors declare that there is no any conflict of interest for this manuscript

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Short Communications

Fertility and fecundity rate in progesterone impregnated intravaginal sponge and eCG administered non-descript goats

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ABSTRACT

The efficacy of estrus synchronization for improving the fertility and fecundity was studied in 428 non-descript goats in Kanchipuram district (Tamil Nadu) selected based on clinical and ultrasound examination for synchronization. Non pregnant does were inserted with intravaginal progesterone sponge containing 300 mg of progesterone on day 0 and administered Inj. Cloprotenol sodium (Pragma) 125µg on day 10. The sponge was removed on day 11 of insertion followed by intramuscular injection of 200 I.U. of eCG (Inj.Folligon). Timed Artificial Insemination was done twice at 12 and 24 h interval using Tellicherry frozen semen. Among the synchronized does,359 does were confirmed pregnant by 30-45 days post insemination using transrectal ultrasonography with a conception rate of 83.87% and fecundity rate of 2.038. The study concludes that the progesterone based intravaginal sponge in combination with eCGcan be effectively used for estrus synchronization programme to improve conception rate and fecundity in non-descript goats at farmers flocks.

Key Words: Fertility, Fecundity, Goats, Intra-vaginal progesterone device, eCG

INTRODUCTION

In the last decades, synchronization protocols are based on controlled internal drug release (CIDR) or intravaginal polyurethane sponges impregnated with progesterone (P_4), or their synthetic analogues (progestogens) mainly medroxyprogesterone, melengestrol and fluorogestone acetate forms, along with equine chorionic gonadotropin (eCG) and prostaglandin $F_2\alpha$ ($PGF_2\alpha$) or even estrogenic pharmacologic active substances are found to be effective in small ruminants (Abecia *et al*., 2011).

Combination of progesterone with eCG stimulates the ovarian follicular growth and circulating estradiol levels thereby improving the synchronization

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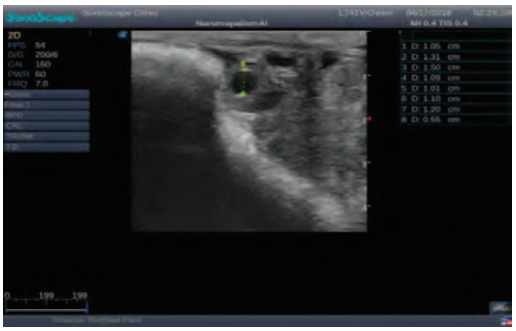
and estrus response rate in both cyclical and non-cyclical females (Quintero-Elisea *et al*., 2011; Lone *et al*., 2016). Under this background, the present study was conducted to investigate the efficacy of estrus synchronization by using progesterone impregnated intravaginal sponges and fixed time artificial insemination (FTAI) protocol in improving conception rate and fecundity rate in non-descript goats.

MATERIALS AND METHODS

The present study was conducted on non-descript goats in 15 selected villages of Kanchipuram district in Tamil Nadu, India. A total of 1556 non-descript goats were screened and 428 goats were selected for further studies.Apparently healthy multiparous goat of 2-6 years of age having approximate body weight ranging from 20-35 kgs of first to fourth parity were having Body Condition Score (BCS) of more than 2.5 andfree from other infections were randomly selected. Pregnant goats and goats with poor body score were not included in this study. The selected does were dewormed with a single dose of Fenbendazole suspension @ 5mg/kg body weight and supplemented with TANUVAS mineral mixture @ 10g/day orally for one month prior to start of study (Fig. 1). Re-examination was carried out after one month using real time Ultrasonography (Sonascape S2V, China) to rule out early pregnancy (Fig. 2a and2b). Then the does were inserted with Intravaginal progesterone Sponge (ICAR-CSWRI, Avikanager, India) containing 300mg of progesterone (Fig. 3 and 4) as per standard procedure and marked as day 0.The animals were given Inj. Cloprostenol sodium (Inj.Pragma, Intas, India) total dose of 125 µg on day 10.The sponge was removed on day 11 of insertion and 200 I.U. of eCG (Inj.Folligon, Intervet, Canada) was administered intramuscularly on the same day. Timed Artificial Insemination was done twice at 12 hours interval with Tellicherry breed frozen semenonday 13 (Fig. 5).

Fig. 1. Deworming of selected goats



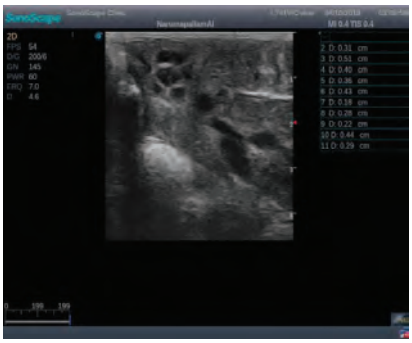


Fig. 2a&2b. Ultrasonic view of ovaries with multiple follicles in non -pregnant doe

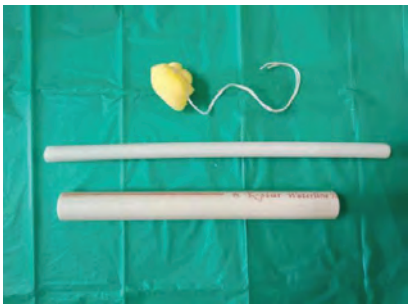


Fig. 3. Intravaginal progesterone sponge with applicator

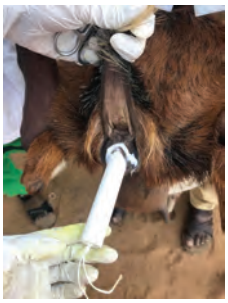


Fig. 4.Intravaginal progesterone sponge insertion

Fig. 5. Artificial insemination using Tellicherry frozen semen

Pregnancy verification was done on 30-45 days post insemination using transrectal ultrasonography (Fig. 6 and 7) and pregnancy was confirmed based on visualization of either gestational sac or concave or C-shaped placentome (Fig. 8a and8b). All data were collected and analyzed. The conception rate was calculated as number of does confirmed pregnant to the number of does mated and the fecundity rate was calculated as the number of kids born to the number of does mated.

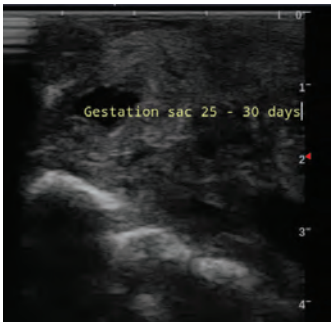


Fig. 6. Pregnancy diagnosis- Gestational sac and multiple fluid pockets

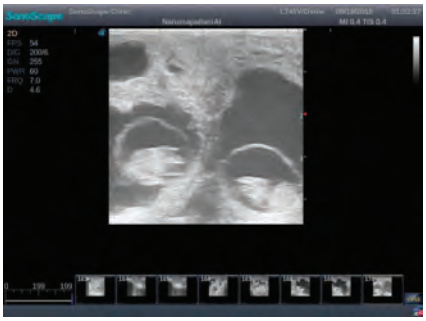
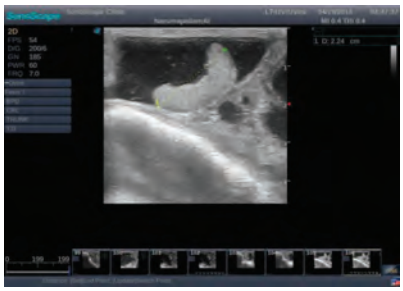


Fig. 7. Pregnancy diagnosis- Gestational sacs of twin fetuses



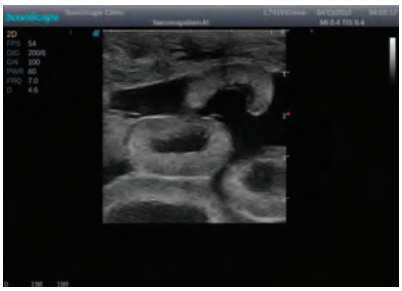


Fig. 8a&8b. Concave or C- shaped placentome

RESULTS AND DISCUSSION

In initial screening out of 1556 goats, 21 (1.35%) were repeat breeders, 678 (43.57%) were pregnant, 77 (4.95%) were in estrum, 204 (13.11%) had poor body condition (Table 1). In the present study, all the animals (100%) responded for estrus synchronization and exhibited estrus and all the does were inseminated with Tellicherry breed frozen semen. The present study shows that out of 428 animals synchronized, 359 goats were tested positive for pregnancy with a conception rate of 83.87%. Furthermore, 63 (17.55%) does delivered singleton, 219 (61%) does delivered twins and 77 (21.44%) does delivered triplets (Table 2).

Table 1: Does screened for Estrus synchronization protocol under field condition at Kanchipuram district

S. No.	Attributes	Total and percentage
1	No. of animals screened	1556 (100%)
2	No. of animals pregnant at the time of initial screening	678(43.57%)
3	No. of animals with poor body condition	204 (13.11%)
4	No. of animals at estrum	77 (4.95%)
5	No. of repeat breeder	21 (1.35%)
6	No. of animals with metritis	6 (0.38 %)
7	No. of hermaphrodite animals	1 (0.064%)
8	No. of animals selected after initial screening	569 (36.56%)
9	No. of animals pregnant at second screening	141 (24.78%)
10	No. of animals selected for estrus synchronization after second screening	428 (75.22%)

Table.2. Effect of intravaginal progesterone device and timed AI on conception rate in non-descript goats

S. No.	Attributes	Details
1	No. of animals screened	1556
2	No. of animals synchronized	428 (100%)
3	No. of animals responded for estrus synchronization and percentage	428 (100%)
4	No. of animals AI done	428 (100%)
5	No. of animals confirmed pregnancy	359 (83.87%)
6	Conception rate	83.87%
7	No. of kids born	732
	a) No. of animals kidded single kid	63 (17.55%)
	b) No. of animals kidded twins	219 (61%)
	c) No. of animals kidded triplets	77 (21.44%)
8	Fecundity rate	2.038

In the present study, the estrus response following progesterone and eCG treatment was observed to be 100%. This finding is in line with the reports of Luther *et al*. (2007) and Kavitha *et al*., (2018) who also reported estrus response of 100%, using progesterone intravaginal sponge whereas De *et al*., (2015) reported a lesser estrus response of 79.4% using AVIKESIL sponges in ewes. The progesterone impregnated intravaginal sponges act as an artificial corpus luteum and elevated the progesterone level in circulation upon its insertion thus suppressing the pulsatile release of GnRH and LH and arrest the follicular activity. Consequently, the drop

in circulatory concentration of progesterone after progesterone implant withdrawal promote the release of GnRH, followed by FSH and LH release leading to resumption of ovarian cyclicity (Zerbe *et al*., 1999). Therefore, combination of progesterone impregnated intravaginal device, $PGF_2\alpha$ and eCG enhances the intensity of estrus behavior and eCG also increases the fecundity rate.

Vinoles *et al*., (2001) recorded 67.0%, in ewes synchronized with MAP intravaginal sponge *in situ* for 12 days followed by eCG injection and only 60.0% conception rate reported by Martemucci and D'Alessandro (2010) in ewes synchronized with 40 mg of FGA intravaginal sponges *in situ* for 14 days followed by 400 IU of eCG at the time of sponge withdrawal. However, Dogan *et al*.,(2018) reported a lower conception rate (27.8%) using norgestomet ear implants plus an intramuscular injection of 500 IU of equine chorionic gonadotropin (eCG) and 125 µg cloprostenol ($PGF_2\alpha$), 48 h prior to progestagen removal.

The conception rate in the present study was recorded to be 83.87% which was higher as compare to previous reports indicating that the progesterone based intravaginal device for estrus synchronization programme and Timed Artificial Insemination protocol along with eCG treatment were effective in improving the conception rate and fecundity rateto 83.87% and 2.038 respectively. Hence, present study concludes that progesterone impregnated intravaginal sponge combined with eCG could be effectively employed for Timed insemination and to improve the conception rate and fecundity rate economically.

Hence upgrading the local goat breeds with 3 kidding in every two years under field conditions can thus be achieved by hormonal interventions.

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Persistent hymen with mucus accumulation in a crossbred heifer

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Hymen normally appears as a circular constriction between vagina and vulva. However varying degrees of persistence, from thin band to complete imperforate structure, may occur in all species due to segmental defects of paramesonephric ducts (Roberts, 1971). Complete obliteration of vaginal canal due to imperforate hymen was reported earlier in crossbred (Kumar *et al*., 2017), pure bred (Kumar *et al*., 2020) and buffalo heifers (Singh *et al*., 2010 and Kumar *et al*., 2016) with varying degrees of mucus accumulation. The present report places on record a case of persistent hymen with voluminous mucus accumulation in a crossbred heifer.

Case history and Clinical Observations

A Jersey crossbred heifer (2.5 years) was referred to the Gynaecology section of Veterinary Clinical Complex, Veterinary College and Research Institute, Orathanadu, Tamil Nadu with the history of urinary incontinence, in appetence and straining for the past one week after being inseminated by a quack. Anamnesis revealed that animal was exhibiting oestrus signs without vaginal discharge and was inseminated thrice previously.

Per rectal examination revealed a massive enlarged structure extending from the vaginal region to the level beyond the pelvic brim. The texture of the structure did not resemble that of pregnant uterus. Cervix was not palpable and hence suspected of abnormality in urinary bladder based on the history. There was no reduction in the mass even after emptying the urinary bladder by catheterization.

Exploration of vagina by speculum revealed a complete obliteration of vaginal canal beyond the urinary meatus, about 11 cm from the external genitalia (Fig.1). Based on the observations, the condition was diagnosed as 'Imperforate or Persistent Hymen' as per the classification of Roberts (1971). Two punctured points could be observed in the middle of the hymen which would have been caused by the faulty inseminations.

Ultrasonographic investigation with trans-rectal probe (Sonoscape S2V, China) was carried out which revealed a massive accumulation of fluid with echoic spots (resembling purulent debris) extending from vaginal region posteriorly to uterine horns anteriorly (Fig.2). Cervix was difficult to distinguish as it was extensively dilated with fluid.

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Treatment and Discussion

Vaginal speculum was passed and held in position against the septum. A sterile intrauterine catheter was passed through the septum and thick cloudy mucus started flowing out following the catheterization. To facilitate easy and complete evacuation, suction pump was employed and about 15 litres of mucus was removed. After the fluid evacuation, per rectal examination revealed that the uterine contours palpable within the pelvic cavity. Ultrasonographic examination showed a clear band of tissue across the vaginal canal posterior to cervical region (Fig. 3a).

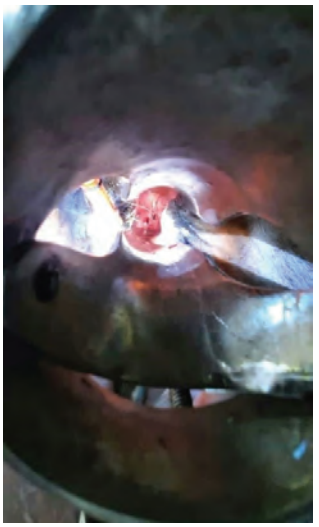


Fig.1:Visualization of hymen through vaginal speculum

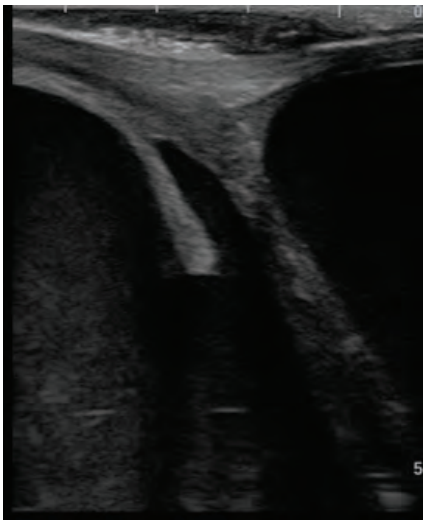
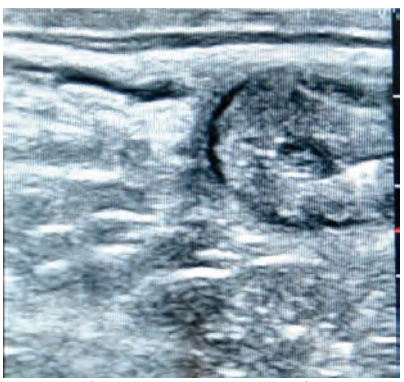
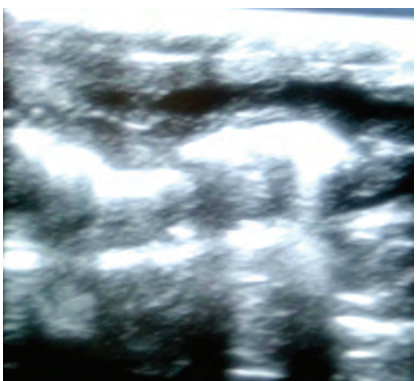


Fig.2: Ultrasonographic imaging of uterus and cervix with accumulated mucus



a. Persistent hymen blocking the vaginal canal



b. Patent vaginal canal

Fig.3: Ultrasonographic images of vaginal passage before and after surgery

The complete blockage of the vaginal canal due to the congenital anomaly resulted in accumulation of uterine and cervical secretions and formation of mucometra, mucocervix and mucovagina as described by Parkinson (2001). Previously Kumar *et al*. (2017) reported only mucocervix and mucovagina in association with persistent hymen in a crossbred heifer. However, Kumar *et al*. (2016) had documented a case of imperforate hymen along with development of pyometra, pyocervix and pyovagina in a Murrah buffalo heifer.

As a correction measure, a circular incision along the entire outer border of the persistent hymen was preferred as described by Roberts (1971). Under epidural anaesthesia, endotracheal tube meant for small animal anaesthesia was inserted through the opening created by the intrauterine catheter. The cuff was positioned anterior to the septum and inflated and thus the tube was fixed in position. The septum was retracted back near the vulvalopening by pulling the tube. Holding the tube in position, circumferential resection of the septal tissue was performed using the BP blade. The incised wound was cleaned and vaginal canal was douched with potassium permanganate solution. Emollient of povidone iodine and cetrimide cream were applied around the entire vaginal canal. A surgical gauze tampon smeared with streptopenicillin powder was inserted and positioned at the site of incision to prevent adhesion. Antibiotics (Inj. Streptopenicillin 5gm; im), antihistaminics (Inj. Chlorpheneramine maleate 8 ml; im) and anti-inflammatory (Inj. Flunixin 7 ml; im) drugs were administered for five days. Seven days post-surgery, the tampon was removed. Examination revealed a free passage of vaginal canal (Fig.3b) and satisfactory healing of the incised site. The uterine horns were empty and totally devoid of mucus accumulation. Sexual rest was recommended for two cycles.

Previous reports documented evacuation of 800ml to four litres of accumulated mucus in similar cases of imperforate hymen (Singh *et al*., 2010, Kumar *et al*., 2016, 2017 and 2020) but in the present case, around 15 litres of accumulated mucoid fluid was evacuated which indicated the chronic nature of the condition. The voluminous accumulation of the mucus was due to the cyclical secretions of previous estrus cycles.

Urinary incontinence might be due to obstruction of the urethra caused by the enlarged reproductive tract. After evacuation of the genital contents, urination was restored to normalcy.

The report documented the incidence of persistent hymen associated with massive accumulation of mucus and its successful correction in a crossbred heifer.

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