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The Editor
Journal of Veterinary and Animal Sciences
College of Veterinary & Animal Sciences
Mannuthy - 680651, Thrissur, Kerala, India
+91- 487- 2370344 ext. 228; 334
Mob.: +91 9895268573
Fax No: +91 487 2370344
e mail : *editorvetj@kvasu.ac.in*
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Hyperthyroidism in cats: An overview

G. Ajitkumar[1] and R. Praseeda[2]
Deerfoot South Spay, Neuter and Wellness Clinic Calgary, Alberta, Canada

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Abstract

Hyperthyroidism, primarily due to functional thyroid adenoma, is the most common endocrinopathy of older cats worldwide. Even though the etiopathogenesis of feline hyperthyroidism is not yet fully understood, the four common therapeutic modalities that can be implemented individually or in combination for management are surgical thyroidectomy, radioactive iodine, pharmaceutical therapy and dietary therapy using a limited-iodine diet. Regular monitoring of a hyperthyroid cat is important to assess therapeutic efficacy, to detect iatrogenic hypothyroidism and to confirm comorbidities that become evident with resolution of the hyperthyroidism.

Thyroid gland is an endocrine gland located at the neck area. In cats, this gland has two lobes connected by an indistinct isthmus. The functional unit of the gland is the thyroid follicle and the three hormones produced are triiodothyronine (T3), thyroxine (T4) and calcitonin. Secretion of T3 and T4 are regulated by the thyroid-stimulating hormone (TSH) and thyrotropin-releasing hormone (TRH) produced by the pituitary gland and hypothalamus, respectively. Ectopic or accessory thyroid tissue is relatively common in many species including cats and may be located anywhere from the larynx to the diaphragm. Hyperthyroidism is characterized by the overproduction of thyroid hormone and a subsequent increase in metabolic rate.

Incidence

The most common endocrinopathy in cats is hyperthyroidism. It became evident first about four decades ago (Peterson *et al.*, 1979; Holzworth *et al.,* 1980). The prevalence of feline hyperthyroidism has steadily increased worldwide since those first reports and is now diagnosed in 1.5 to11.4 per cent of older cats around the world (Miyamoto *et al.*, 2002; Wakeling *et al.*, 2005; Sassnau, 2006; De Wet *et al*., 2009; Peterson, 2012). Among middle-aged or older cats in the United States of America, its prevalence is up to 10 per cent (Peterson, 2012; Vaske *et al*., 2016).

The most common pathologic abnormality associated with hyperthyroidism in cats is functional thyroid adenoma involving one or both thyroid lobes (Holzworth *et al.,* 1980; Hoenig *et al.*, 1982; Gerber *et al.*, 1994). Most hyperthyroid cats have bilateral disease and studies indicated that removal of a functional adenoma might be followed by the development of a contralateral

1. *Chief Veterinarian. Email id: ajitvet@yahoo.co.in Deerfoot South Spay, Neuter and Wellness Clinic, Calgary, Alberta, Canada.*
2. *Associate Veterinarian*

one. Scintigraphic evidence suggested that the adenoma could continue to grow if ablative surgery or radioiodine were not chosen for management of the initial mass (Peterson, 2012; Peterson and Broome, 2012; 2015). Malignant carcinoma was found at the time of initial diagnosis only in two per cent of hyperthyroid cats (Turrel *et al.,* 1988; Hibbert *et al*., 2009).

Etiopathogenesis

A clear picture of the various causes of hyperthyroidism in cats is not yet fully understood. Multiple factors play a role, but the relative importance of each is unknown (Scarlett *et al*., 1988; Kass *et al*., 1999; Wakeling *et al*., 2009; 2011). Genetics may influence susceptibility to hyperthyroidism. In one study, Siamese and Himalayan breeds were found to have diminished risk of developing hyperthyroidism (Kass *et al*.,1999). Changes in cat husbandry over the past several decades, including a higher percentage of indoor cats, increased utilization of commercial cat foods and longer lifespans, may influence the prevalence (Scarlett *et al*., 1988; Kass *et al*., 1999; Wakeling *et al*., 2009).

The occurrence of bilateral thyroid disease strengthens the hypotheses of dietary and environmental etiologies than mutational causes alone (Hammer *et al*., 2000). Lifelong exposure to environmental thyroid disruptor chemicals or goitrogens through food or water may lead to euthyroid goiter and then to autonomous adenomatous hyperplasia, thyroid adenoma and hyperthyroidism (Peterson, 2012). Epidemiologic studies have identified a list of compounds like phenols and halogenated hydrocarbons having association with the incidence. The use of deodorized litter material and/or canned food that may contain bisphenol A and phthalates also have been associated with feline hyperthyroidism (Kang and Kondo, 2002; Edinboro *et al*., 2004; Peterson, 2012). Soy isoflavones, a component of many cat foods and the common environmental contaminant fire-retardant polybrominated diphenyl ethers may act as goitrogens via TSH stimulation or as direct mitogens (Court and Freeman, 2002; Guo *et al*., 2012; Mensching *et al*., 2012; Norrgran *et al*., 2012). Variable iodine content of cat foods also seems to have an influence on the development of the disease (Edinboro *et al*., 2010; 2013; Wedekind *et al*., 2010). So far, prospective evaluation of the effect of lifelong exposure to a specific compound in development of hyperthyroidism in cats has not been studied in detail and hence all associations are assumptions (Carney *et al*., 2016). A recent study to identify associations between coat phenotype and feline hyperthyroidism by investigation of breed, coat colour and hair length as risk factors, identified decreased risk of hyperthyroidism in the Tonkinese, Abyssinian and British shorthair breeds. It also identified an association between risk of hyperthyroidism and hair length and confirmed decreased risk in Burmese, Siamese, and Persian breeds (Crossley *et al*., 2017).

Clinical symptoms

The common signs of feline hyperthyroidism include progressive weight loss in spite of a ravenous appetite (polyphagia), polyuria, polydipsia, increased vocalization, aggressive behavior, agitation, restlessness, hyperactivity, tachypnea, tachycardia, periodic vomiting, diarrhea, weakness, depression and a poor unkempt hair coat (Bruyette, 2013; Carney *et al*., 2016).

Diagnosis

Diagnosis of feline hyperthyroidism is based on presence of one or more clinical signs and increased serum total T4 concentration. However, up to 10 per cent of all hyperthyroid cats and 40 per cent of those with mild disease have serum T4 values within the reference range (Broussard *et al*., 1995; Peterson *et al*., 2001). Hyperthyroidism should not be excluded based on a single normal serum T4 value especially in a cat with typical clinical signs, a palpable thyroid nodule and serum T4 in the upper half of the reference range (Peterson *et al*., 1987). In these cases, serum free T4 (fT4) measured by equilibrium dialysis may provide an alternative approach to diagnosis. Studies document that up to 20 per cent of sick euthyroid cats can have increased fT4 concentrations (Wakeling *et al*., 2008). Therefore, it is appropriate and reliable to interpret the total T4 and fT4 values together. Mid to high serum total T4 (grey zone)

and increased fT4 concentration are consistent with hyperthyroidism. A definitive diagnosis of feline hyperthyroidism requires demonstration of persistently elevated thyroid hormone concentrations (T4, or T4 plus fT4) occurring concurrently with one or more of the typical clinical signs. Differential diagnoses for cats with clinical signs similar to hyperthyroidism should include diabetes mellitus, gastro-intestinal malabsorption or maldigestion, neoplasia, chronic kidney disease and parasitism (Carney *et al*., 2016).

Management options

Being a life-threatening disease, hyperthyroidism in cats requires prompt veterinary attention. The choice of therapy often depends on factors such as age of the affected cat, comorbidities like renal, heart disease and hypertension, cost of the treatment, availability of treatment options and the clinician's recommendations and expertise. The goal of therapy is to restore euthyroidism, avoid hypothyroidism and minimize side effects of treatment. As a rule, treat all cats diagnosed with hyperthyroidism and monitor them prudently (Carney *et al*., 2016).

In general, there are four methods for managing feline hyperthyroidism. One is a permanent solution through thyroidectomy. This has more than 90 per cent cure rate if both glands are removed and 35 to 60 per cent cure rate if one gland is removed. It cures disease within one to two days post-surgery and the relapse rate is about five per cent in bilateral procedure and 30 per cent in unilateral procedure. The disadvantages of surgical thyroidectomy include increased risk involved in general anesthesia of a cat with a compromised cardiovascular system, damage to parathyroid gland and subsequent transient or permanent calcium crisis. The procedure requires hospitalization, is not reversible and most hyperthyroid cats require stabilization first with medication prior to the surgery. The voice or purr of the cat may change after the surgery (Carney *et al*., 2016).

The gold standard treatment for feline hyperthyroidism is radioactive iodine. After administration, it is actively concentrated by the thyroid gland, with a half-life of eight days. Even though, it emits both beta-particles and gamma-radiation, the beta-particles are responsible for most of the local tissue destruction traveling a maximum of two millimeter. Therefore, no significant damage to adjacent parathyroid tissue, atrophic thyroid tissue, or other cervical structures is expected (Bruyette, 2013). It kills abnormal cells in any locations and the cure rate is more than 95 per cent. This is the most successful treatment for thyroid carcinoma and the relapse rate is only five per cent. The treatment is simple consisting of one injection or oral capsule and serious side effects are rare. Limited testing is needed after successful treatment with minimal risk of permanent hypothyroidism. The main limitation to widespread use of radioactive iodine is the requirement for special licensing from the local regulatory authority and isolation of the cat after treatment, which can range from several days to several weeks depending on local radiation regulations and the dose administered. (Mooney, 2010; Carney *et al*., 2016).

Medical management of hyperthyroidism through daily administration of antithyroid medications *via* oral or transdermal route is the most widely used. These drugs can be used long term as a sole treatment or short term to stabilize the patient before any surgery or anesthesia or if radioiodine therapy is not immediately available (Mooney, 2001; Trepanier, 2007). Two pharmacologically active ingredients are available as licensed veterinary drugs for treatment of hyperthyroidism, methimazole and carbimazole (Carney *et al*., 2016). Methimazole acts by blocking thyroid peroxidase, thus inhibiting biosynthesis of thyroid hormones (Trepanier *et al*., 1991). Methimazole is thought to accumulate in the thyroid glands of cats as in humans (Trepanier *et al*., 1991; Okuno *et al*., 1987). Oral methimazole is well absorbed in healthy cats and the pharmacokinetic parameters are not significantly altered by hyperthyroidism (Trepanier *et al*., 1991). Carbimazole is a metabolite of methimazole that has a similar mechanism of action as well as side effects (Frénais *et al*., 2009). The most severe, but rare, side effects observed with

methimazole are hepatopathy and marked blood dyscrasias such as severe leucopenia, anemia and thrombocytopenia. Gastro-intestinal upset, lethargy and facial pruritus occur at variable frequency. Occurrence, frequency and severity of side-effects have not been shown to be dose related (Peterson *et al*., 1988; Hill *et al*., 2011). Gastro-intestinal upset may be less frequent with transdermal preparations (Hill *et al*., 2011). Most side effects appear within the first four to six weeks of therapy and are less common after two or three months of treatment (Peterson *et al*., 1988). The advantages of medical management include a response rate of more than 95 per cent while on medication in the form of small pills, liquid or topical gel. This does not require hospitalization and there is no risk of permanent hypothyroidism. Medication can be discontinued or dosage can be reduced if kidney function declines. Disadvantages include a relapse rate 100 per cent when the medication is off, daily medication for the rest of the cat's life and frequent laboratory tests to monitor effectiveness and safety. Drug reactions in the form of facial itching, vomiting, liver failure, abnormal blood cell levels and bleeding episodes may occur in up to 25 per cent of cats. Even with the treatment, the tumour continues to grow and may become malignant (Carney *et al*., 2016).

Limited iodine diets can also be given to normalize thyroid hormone concentrations and alleviate clinical signs of hyperthyroidism. Production of thyroid hormone requires sufficient dietary iodine. The only known function for ingested iodine is for thyroid hormone synthesis (Peterson, 2008). This observation led to the hypothesis that limiting dietary iodine intake could be used to control thyroid hormone production and potentially manage hyperthyroidism in cats. This option is simple and straightforward requiring only a change in diet. The response rate is more than 82 per cent while on the diet and is safe in cats with renal insufficiency. Absolutely the only food the cat can eat for the rest of its life will be limited iodine diets and has to be fed with low iodine treats and water as well. The relapse rate is cent per cent when the cat is off the special diet (Carney *et al*., 2016).

Conclusion

Hyperthyroidism, the most common endocrine disorder in cats, first became evident about four decades ago. Its prevalence has steadily increased over the past years reaching about 10 per cent among the older cat population. Even though, thyroid adenoma involving one or both lobes being the most common pathologic abnormality associated with feline hyperthyroidism, the etiopathogenesis is not yet fully understood. Being a life-threatening disease in cats, it requires prompt veterinary attention. Surgical thyroidectomy, radioactive iodine treatment, pharmaceutical therapy and dietary therapy using limited-iodine diet are the four common therapeutic modalities that can be implemented individually or in combination for management of feline hyperthyroidism.

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RESEARCH ARTICLE
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Effect of jackfruit powder on the physicochemical and sensory attributes of poultry meat cocktail nuggets*

Silpa Sasi[1], T. Sathu[2], C. Sunanda[3], M. Pavan[4], V.N. Vasudevan[2], A. Irshad[2], and S. Kiran Kumar[1]

Dept. of Livestock Products Technology and Meat Technology Unit College of Veterinary and Animal Sciences, Mannuthy, Kerala - 680 651. Kerala Veterinary and Animal Sciences University

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Abstract

The present study was designed to investigate the effect of jackfruit powder on the physicochemical characteristics, proximate composition and sensory attributes of cocktail nuggets containing 75 per cent chicken and 25 per cent duck meat. Jackfruit powder was added in cocktail nuggets at three different levels i.e., one, two and three per cent over and above the cocktail nuggets formulation and its effect was evaluated against control cocktail nuggets. There was no significant difference in emulsion pH values of control and jackfruit powder incorporated treatment samples. When compared to control nuggets, significant increase was noticed in the product pH of all the three treatment nuggets incorporated with jackfruit powder. No significant difference was observed in the water activity and cooking yield of treatment and control nuggets samples. No significant difference was observed in the moisture, fat, carbohydrate and calorie content of the jackfruit powder added treatments and control nuggets. Protein percentage of treatment samples were significantly lower than the control nuggets. Significantly higher ash content was observed for jackfruit powder incorporated treatment samples when compared to control samples. On sensory evaluation, no significant difference was observed for the appearance and flavour between control and the treatment samples. Functional cocktail nuggets containing three per cent jackfruit powder had significantly lower values for juiciness, texture, saltiness, mouth coating and overall acceptability when compared to other treatment and control samples. The addition of jackfruit powder in the nugget formulations was effective in sustaining the desired sensory attributes besides the nutritional benefits. Hence, acceptable functional cocktail nuggets can be made with the addition of jackfruit powder up to two per cent over and above the cocktail nuggets formulation without affecting the sensory attributes.

Keywords: *Cocktail nuggets, Jackfruit powder*

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Correspondence E-mail: silpasasi2011@gmail.com, Ph : 8137958806

1. *PG Scholar, Department of Livestock Products Technology*
2. *Assistant Professor, Department of Livestock Products Technology*
3. *Assistant Professor, Department of Statistics*
4. *Ph.D. Scholar, Department of Livestock Products Technology*

The relationship between food and human health has been studied since time immemorial. The trends in production and consumption of food is closely related to human health and wellbeing. With the development of newer technologies, an increasing number of beneficial food products with added functional ingredients have gained an important place in the global market. Functional foods, can be defined as those foods that are fortified, enriched, or enhanced with bioactive ingredients that may reduce the risk of diseases and can provide added physiological benefits. Functional foods claim to improve health and help prevent certain diseases when combined with balanced diet and healthy lifestyle.

The consumption of jackfruit has increased due to its reported health benefits. Jackfruit is a rich source of various bioactive components which makes it a favorite subject for the scientific community. However it still remains underutilized in terms of production of value added food materials. The use of standardized meat products incorporated with jackfruit can be considered as a novel way for reaping the health benefit properties of the fruit.

Keeping in view all the above facts, the present study was envisaged to attempt the still inconclusive studies on utilization of jackfruit powder in the development of functional meat products. A study was designed to evaluate the effect of different levels of jackfruit powder on physicochemical properties, proximate composition and sensory profile of cocktail nuggets containing 75 per cent chicken and 25 per cent duck meat.

Materials and methods

Chicken and duck meat

Broiler chicken and duck each of 2.5 to 3 kg live body weight procured from the local market were humanely slaughtered and dressed under hygienic conditions at Meat Technology Unit, Mannuthy. The dressed carcasses were immediately chilled for around 24 hours and deboned. Deboned meat was aerobically packed in high density polyethylene (HDPE) bags, kept frozen and thawed at 4±1°C before the preparation of nuggets.

***Vegetable Oil*:** Refined sunflower oil (Sunrich) was used throughout the study.

Condiment: The condiment mixture was prepared as and when required by blending peeled and chopped onion and garlic (3:1 w/w) to the consistency of a fine paste.

Spice mixture: consists of coriander 22%, cumin seeds 16%, black pepper 20%, red chilli 7%, anise 5%, dry ginger 5%, turmeric 5%, cinnamon 5%, cardamom 5%, curry leaves 2%, clove 2%, nutmeg 3% and mace 3%.

Curing ingredients: Sodium chloride 1%, sugar0.3%, sodium-tri-polyphosphate 0.3%, sodium ascorbate 550 ppm and sodium nitrite 120 ppm.

Functional ingredient: jackfruit powder (Jackfruit 365, Eastern Condiments Pvt Ltd) purchased from local market of Thrissur, Kerala was used throughout the study.

Product formulation

The formulation of emulsion based cocktail nuggets was standardized by conducting several trials. The standardized formulation was used for the entire study (Table.1).

Preparation of chicken nuggets

Deboned broiler chicken was minced through a 9 mm grinder plate in a meat mincer (MADO primus Model MEW 613, Germany). The ground chicken was pre-blended with salt, sodium tripolyphosphate, sugar, and sodium ascorbate and sodium nitrite at the levels given in the Table 1 and kept under refrigeration for about 12 hours. The emulsion was prepared in a bowl chopper (MADO GARANT, Germany) by chopping the pre-blended chicken for 3-5 min with simultaneous addition of ice flakes. Beaten egg was added and chopped further for 1-2 min, followed by the addition of pre-chilled refined sunflower oil till it was evenly dispersed in the batter during chopping. Then, binders, corn flour and refined wheat flour (1.5%) each, soya powder (2%), condiments (4%)

Table 1 Formulary for the preparation of control and functional cocktail nuggets

Ingredients (%)	Control nuggets (%)	Functional cocktail nuggets
Ground chicken	50.625	50.625
Ground duck meat	16.875	16.875
Ice flakes	10	10
Vegetable oil (sunflower oil)	12	12
Condiments	4.0	4.0
Spice mix	1.7	1.7
Soya powder	2.0	2.0
Corn flour	1.5	1.5
Refined wheat flour	1.5	1.5
Salt	1.0	1.0
Sugar	0.3	0.3
Sodium tripolyphosphate	0.3	0.3
Sodium ascorbate	0.3	0.3
Sodium nitrite	120 ppm	120 ppm
Egg	3.33	3.33
Jackfruit powder	0.0	*

***jackfruit powder** was added over and above the quantity of the formulation at three different levels.

and spices mix (1.7%) as per formulary were added. Jackfruit powder was added with the mix and chopped till uniformly dispersed with desired consistency of the batter. The batter was taken and manually filled in stainless steel mould under hygienic condition. The mould covered with lid was steam cooked for 40 minutes to get properly cooked blocks. Chicken blocks so obtained were cooled and kept under refrigeration for 12-15 hours. These blocks were sliced into nuggets of size 1.5 cm x 1.5 cm x 1.5 cm. The product preparation procedure for different chicken nuggets formulations was uniform throughout the study.

Analytical procedures

Physico-chemical characteristics

pH

The pH of the chicken nuggets from all the treatments and control, before and after cooking was determined using a combined electrode digital pH meter (μ pH system 362, Systronics, India) as per procedure of Troutt *et al*. (1992).

Water activity (a_w)

For determination of a_w, the samples were cut into small pieces and filled in the sample cup up to the mark. The filled sample cup was kept in the measurement chamber of Lab swift a_w meter (Novasina, Switzerland). The readings were taken, when the stable a_w was on in the display.

Cooking yield percentage

The weights of meat loaves before and after cooking were recorded. Product yield was expressed in percentage.

$$\text{Product yield (\%)} = \frac{\text{weight of cooked meat block}}{\text{Weight of raw batter}} \times 100$$

Proximate analysis

The proximate composition of the chicken nuggets batter and products were determined by the standard procedure of AOAC (2016). Analyses were conducted in duplicate.

Moisture was determined by weight loss after 16 hours drying in a hot air over at

105°C. The fat content was determined in moisture free samples by an ether extraction procedure in an Automatic Solvent Extraction System (SOX plus, Model SCS 6, Pelican Equipments, Chennai, India). Moisture and fat free samples were used to estimate the protein and ash content. The protein content was determined by Block Digestion Method (KEL plus, Model KES 6L, Pelican Equipments, Chennai, India). Ash was determined by weight loss after 2 hours drying in muffle furnace (HF-electric furnace, Hindustan Furnance, Thrissur, Kerala) at 600°C. The amount of carbohydrate was calculated as 100 minus sum of the percentage of moisture, protein, fat and ash. The proximate composition was expressed in as-is-basis.

Calorific Value

Total calories content of chicken nuggets were arrived at as per FAO (2002) on wet matter basis.

Calories from fat = fat per cent × 9
Calories from protein = protein per cent × 4
Calories form carbohydrate = carbohydrate per cent × 4.
Total calories (kcal/100g) = (fat% x 9) + (protein% x 4) + (carbohydrate % x 4).

Organoleptic evaluation

Sensory attributes of the chicken nuggets were assessed organoleptically using 8 point Hedonic scale score card (AMSA, 1983) with the help of seven semi-trained taste panellists drawn from the Department of Livestock Products Technology, Mannuthy, Thrissur. The nuggets were shallow fried in sunflower oil and served warm to the panellists with code numbers to the samples. The average of the individual scores was taken as the score for the particular attribute.

Statistical analysis

The experiment was replicated four times and the data obtained for physico-chemical and sensory evaluation of different products were statistically analyzed as per Snedecor and Cochran (1994) by one-way ANOVA and Kruskal-Wallis test using SPSS software version 24.

Result and Discussion

The results of physico-chemical characteristics of the functional cocktail nuggets incorporated with different levels of jackfruit powder (one, two and three per cent) are presented in Table 1.

There was no significant difference in emulsion pH values of control (6.80 ± 0.03) and functional cocktail nuggets with three levels of jackfruit powder *viz*. 1 per cent (6.77 ± 0.02), 2 per cent (6.78 ± 0.02) and 3 per cent (6.77± 0.02). When compared to control nuggets, significant ($p< 0.001$) increase was noticed in the product pH of all the three treatment nuggets incorporated with jackfruit powder. Similar to this, Kumar *et al*. (2010) reported a higher pH for the chicken nuggets incorporated with soya hull flour when compared to control nuggets and this increase in pH was correlated to higher pH of the soya hull flour.

No significant difference was observed in the water activity and cooking yield of functional cocktail nuggets containing different levels of jackfruit powder and control nuggets samples. However, Verma *et al*. (2012) stated a marked decline in cooking yield with increase in level of incorporation of chick pea hull flour in low salt chicken nuggets.

Proximate composition and calorie content

The proximate composition of the cocktail nuggets incorporated with different levels of jackfruit powder is shown in Table 2.

There was no significant difference in the moisture, fat, carbohydrate and calorie content among the treatment and control samples. The addition of jack fruit powder had no effect on the moisture, fat, carbohydrate and calorie content content of cocktail nuggets. This was in accordance with Dzudie *et al.* (2002) who observed no significant difference in the moisture and fat content of 2.5 per cent common bean flour incorporated beef sausages in comparison to control sausages.

Protein percentage of treatment samples were significantly ($P<0.001$) lower

Table 2. Effect of jackfruit powder on the physico-chemical characteristics, proximate composition and calorie content of the cocktail nuggets

Parameters	C	T_1	T_2	T_3	F-value (p-value)
Emulsion pH	6.80 ± 0.03	6.77 ± 0.02	6.78 ± 0.02	6.77 ± 0.02	0.274^{ns}(0.843)
Product pH	6.82 ± 0.02^{b}	6.85 ± 0.01^{a}	6.87 ± 0.01^{a}	6.85 ± 0.01^{a}	4.511^{*}(0.014)
Water activity	0.92 ± 0.00	0.92 ± 0.00	0.92 ±0.00	0.93± 0.00	1.728^{ns}(0.193)
Cooking yield (%)	97.76 ± 0.54	98.21 ± 0.24	97.77 ± 0.33	97.88 ± 0.31	0.334^{ns}(0.801)
Moisture (%)	60.79 ± 0.38	60.84 ± 0.42	60.00 ± 0.50	59.78 ± 0.29	1.800^{*}(0.180)
Protein (%)	18.00 ± 0.31^{a}	16.17 ± 0.16^{b}	16.30 ± 0.23^{b}	16.60 ± 0.19^{b}	13.586^{*}(<0.001)
Fat (%)	12.48 ± 0.54	12.31 ± 0.72	12.88 ± 0.42	12.55 ± 0.74	0.151^{*}(0.928)
Carbohydrate (%)	7.06 ± 0.62	8.86 ± 0.95	8.96 ± 0.53	9.20 ± 0.78	1.778^{ns}(0.184)
Ash (%)	1.66 ± 0.02^{b}	1.82 ± 0.02^{a}	1.86 ± 0.02^{a}	1.87 ± 0.02^{a}	19.737^{*}(<0.001)
Calorie (kcal/100 g)	212.62 ± 2.69	210.89 ± 2.22	216. 10 ±3.77	216.12± 3.31	0.889^{ns}(0.464)

** Significant at 0.01 level; * significant at 0.05 level; ns non- significant at 0.05 level
Means with same superscripts in a row does not differ significantly (P >0.05)
C – Control (nuggets with 75% chicken meat, 25% duck meat without jackfruit powder)
T_1 – Treatment 1 (C+1 % jackfruit powder)
T_2 – Treatment 2 (C +2 % jackfruit powder)
T_3 – Treatment 3 (C +3 % jackfruit powder)

Table 3. Effect of jackfruit powder on the sensory attributes of the cocktail nuggets

Attributes	C	T_1	T_2	T_3	χ^2-value (p-value)
Appearance	7.04±0.11	7.25±0.07	7.16 ± 0.05	7.12± 0.09	2.516^{ns} (0.472)
Flavor	6.88 ± 0.11	7.12±0.09	6.95 ± 0.1	6.82± 0.08	6.379^{ns} (0.095)
Juiciness	6.98 ± 0.1^{a}	7.13 ± 0.07^{a}	6.99 ± 0.09^{a}	6.74 ± 0.09^{b}	9.960* (0.019)
Texture	6.90 ± 0.1^{a}	7.11 ± 0.08^{a}	7.02 ± 0.1^{a}	6.56 ± 0.1^{b}	15.205* (0.002)
Saltiness	7.00 ± 0.12^{a}	7.05 ± 0.10^{a}	6.94 ± 0.11^{a}	6.64 ± 0.09^{b}	10.908* (0.012)
Mouth coating	6.89 ± 0.1^{a}	7.16 ± 0.05^{a}	6.94 ± 0.1^{a}	6.60 ± 0.09^{b}	20.950** (<0.001)
Overall acceptability	6.97 ± 0.08^{a}	7.26 ± 0.06^{a}	7.02 ± 0.09^{a}	6.70 ± 0.09^{b}	19.418** (<0.001)

** Significant at 0.01 level; * significant at 0.05 level; ns non- significant at 0.05 level
Mean with same superscripts in a row does not differ significantly (P >0.05)
C – Control (nuggets with 75% chicken meat, 25% duck meat without jackfruit powder)
T_1 – Treatment 1 (C+1 % jackfruit powder)
T_2 – Treatment 2 (C +2 % jackfruit powder)
T_3 – Treatment 3 (C +3 % jackfruit powder)

than the control nuggets. This might be due to reduction in meat content by the over addition of jackfruit powder that incorporate carbohydrates in the treatment nuggets at the expense of protein content. Similar to this, Sathu (2014) observed significant reduction in protein content of functional chicken nuggets incorporated with oat flour when compared to control chicken nuggets.

Significantly (P<0.001) higher ash content was observed for jackfruit powder

incorporated treatment samples when compared to control samples. This can be attributed to higher mineral content of jackfruit powder. Similar to the present findings Elgasim and Al-Wesali (2000) noticed significant increase in the ash content of beef patties when incorporated with 20 per cent samh (*Mesembryanthemum forsskalei Hochst*) flour.

Organoleptic evaluation

A meat product recognized as a food and consumed with satisfaction, depends extensively on its sensory parameters. The organoleptic qualities of functional cocktail nuggets incorporated with jack fruit powder and control nuggets was evaluated using eight point Hedonic scale. The results are presented in Table 3.

There was no significant difference for appearance and colour and flavour between control and the treatments containing jackfruit powder. Functional cocktail nuggets containing three per cent jackfruit powder had significantly lower values for juiciness, texture, saltiness, mouth coating and overall acceptability when compared to other treatment and control samples. Prinyawiwatkul *et al*. (1997) reported comparable sensory scores for overall acceptability, texture and flavour of the treatment nuggets containing mixture of 2.5 per cent each of fermented cowpea and peanut flours and unfortified control chicken nuggets. Banerjee *et al*. (2012) noticed comparable scores for all the sensory attributes for functional chicken nuggets containing broccoli powder extract and control nuggets sample.

Conclusion

Result of the experiment indicated that, jackfruit powder at three per cent level imparted significant flour taste which had considerable adverse effect on the flavour, juiciness, texture and also overall acceptability. Among all the treatments, two per cent jackfruit powder incorporated cocktail nuggets had sensory scores that was comparable to one per cent jackfruit powder incorporated cocktail nuggets and control chicken nuggets samples. Thus, functional cocktail nuggets with high acceptability and nutritive value could be prepared by incorporating jackfruit powder up to two per cent in the formulation without affecting the sensory attributes. The incorporation of jackfruit powder which is endowed with numerous nutritional and health values, in the emulsion based poultry meat cocktail nuggets would definitely enrich the functional value of the product.

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RESEARCH ARTICLE
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Seroprevalence of leptospirosis in cattle in Mannancherry panchayat of Alappuzha district*

S. S. Sreekutty[1], K. Vrinda Menon[2], C.Latha[3], B. Sunil[4], R. Ambily[5]
Department of Veterinary Public Health, College of Veterinary and Animal Sciences, Mannuthy, Thrissur-680651. Kerala Veterinary and Animal Sciences University

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Abstract

Cattle can act as asymptomatic carrier in leptospirosis and can transmit the infection to other animals and humans either by direct contact or indirectly by contaminating the environment with infected urine. Thus, the role of apparently healthy cattle in the maintenance and transmission of the organism needs to be studied. The present study was conducted in 90 apparently healthy cattle in Mannancherry panchayat of Alappuzha district. Serum samples were collected from cattle and subjected to Microscopic Agglutination Test (MAT) using a battery of serovars. A serum dilution of 1:50 and above was taken as positive. Out of the 90 samples analysed, 47 (52.2 per cent) were found positive. The predominant serovars observed in the study were Grippotyphosa (34.04 per cent), Sejroe (25.53 per cent) and Autumnalis (21.3 per cent). The study revealed the importance of asymptomatic cattle in the maintenance and transmission of the disease.

Key words: Asymptomatic cattle, Alappuzha, Microscopic Agglutination Test, Predominant serovars

Leptospirosis is a worldwide anthropozoonotic disease caused by the pathogenic spirochete bacterium of the genus *Leptospira.* Since warm and humid atmospheric conditions favours the survival of the organism in the environment, it is more prevalent in the tropical and subtropical regions compared to temperate regions (Sperber and Schleupner, 1989). Among domestic animals, leptospirosis in cattle has been linked with economic losses mainly due to decrease in milk yield, abortion, mastitis and infertility. Cattle act as inapparent carrier of leptospires as they can carry the organisms in their renal tubules and can excrete them in urine for months,

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1. *MVSc Scholar, Email id: sreekuttysreekumar810@gmail.com*
2. *Assistant Professor*
3. *Professor and Head*
4. *Professor*
5. *Assistant Professor, Department of Veterinary Microbiology*

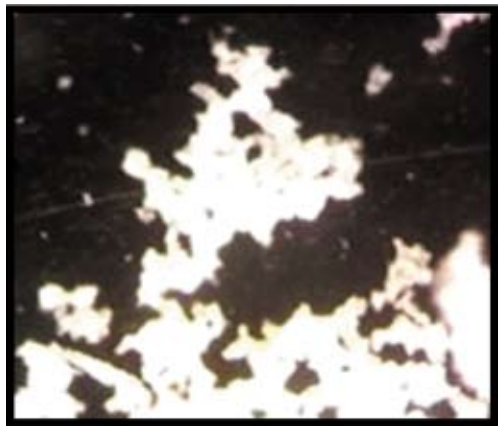
thereby transmitting the infection to other animals and humans through the contaminated environment (Maxie Grant and Newman, 2008). Usually, in maintenance hosts, the infecting serovar may have lower pathogenicity which causes chronic rather than acute infection contrary to accidental hosts (Jose *et al*., 2018).

Even though the disease occurs throughout the year, a higher incidence has been recorded in rainy season because the organism has the ability to sustain in stagnant water, ponds, slow moving water bodies and in slightly alkaline water for a longer time (Levett, 2001). Kerala is highly endemic for leptospirosis which usually suffers post-monsoon outbreaks of the disease among humans and animals (Vijayachari *et al.*, 2008). Knowledge about the common leptospiral serovars circulating in local animal populations is necessary to determine sources and transmission routes for the infection in humans (Hartskeerl *et al*., 2011). The present study aimed at assessing the seroprevalence of leptospirosis in apparently healthy cattle in Mannancherry panchayat of Alappuzha district where higher cattle population and higher incidence of the disease in humans were observed.

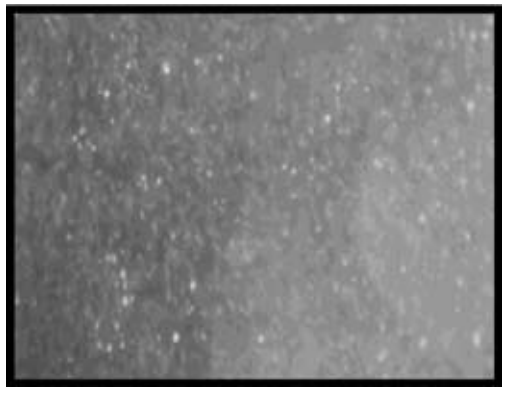
Materials and methods

In the present study, 50 serum samples were collected from apparently healthy cattle of Mannancherry panchayat of Alappuzha district during November, 2018. The samples were stored at -20°C until use. All the serum samples were subjected to Microscopic Agglutination Test (MAT) using a panel of twelve live leptospiral antigens- Australis, Autumnalis, Bataviae, Canicola, Grippotyphosa, Hebdomadis, Icterohaemorrhagiae, Javanica, Pomona, Pyrogenes, Sejroe and Tarassovi. The MAT procedure was performed according to the procedure of Faine (1982) with slight modifications in the final dilution of MAT procedure which was made to 1:50 since the study was done in apparently healthy cattle (Sharma *et al.*, 2006). Further, quantitative assay was carried out against the reacting serovars of leptospires according to Faine (1982) upto a dilution of 1:6400. The reciprocal of the highest dilution of the serum which showed 50 per cent agglutination or 50 per cent reduction in the number of free leptospires in comparison to control was considered as the respective titre.

1:50 dilution

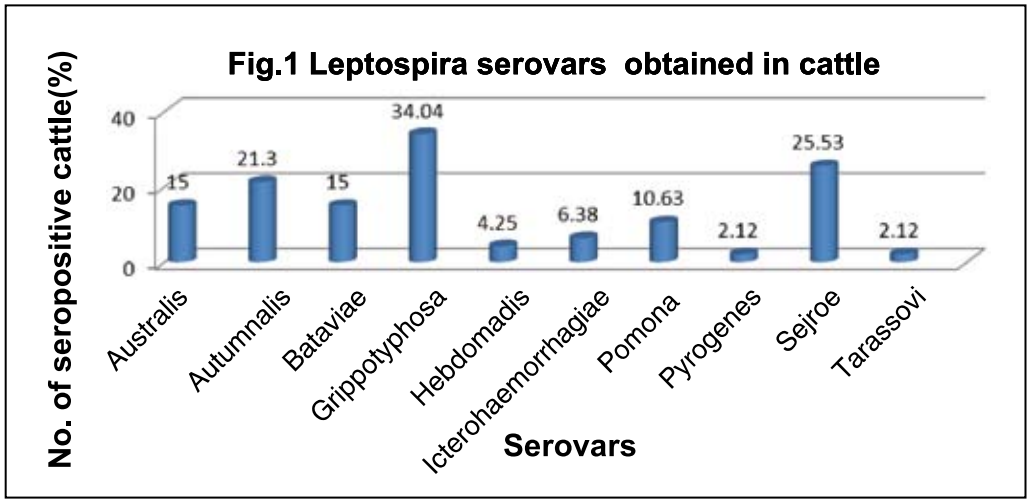


1:100 dilution



Negative control

Microscopic Agglutination Test (MAT) in C



Results and Discussion

All the cattle involved in the study were apparently healthy. Out of the 90 samples analysed by MAT, 47 (52.2 per cent) were found positive where 23 were positive at 1:50 dilution, 17 at 1:100 dilution and seven at 1:200 dilution. The serovars obtained were Grippotyphosa (34.04 per cent), Sejroe (25.53 per cent), Autumnalis (21.3 per cent), Bataviae (15 per cent), Australis (15 per cent), Pomona (10.63 per cent), Icterohaemorrhagiae (6.38 per cent), Hebdomadis (4.25 per cent), Pyrogenes (2.12 per cent) and Tarassovi (2.12 per cent) as shown in Fig.1

In the present study, 52.2 per cent seropositivity was observed in apparently healthy cattle in Mannancherry panchayat at a dilution between 1:50 and 1:200. According to OIE (2018), an antibody titre of 1:100 or more was considered as positive for leptospirosis in animals. However due to high specificity of the MAT, lower titres could be taken as evidence of previous exposure to the organism. Favero *et al.* (2017) stated that even though a titre of 1:100 or higher in the MAT is an indicator of the disease, a titre of 1:50 indicated animal exposure to the etiological agent. Thus, a titre of 1:50 and above was considered as positive in the present study.

The results obtained were in accordance with the study by Soman *et al*. (2014) where a seropositivity of 47 per cent in apparently healthy cattle was observed in Central and North Kerala at a serum dilution of 1:80 and more. Similar study was conducted by Balamurugan *et al*. (2013) where a seropositivity of 48.5 per cent was reported in apparently healthy cattle which had history of abortions and other reproductive disorders. The predominant serovars observed in the present study were Grippotyphosa (34.04 per cent), Sejroe (25.53 per cent) and Autumnalis (21.3 per cent) which was in accordance with the findings of Rani *et al*. (2013) in apparently healthy cattle in Andhra Pradesh where the predominant serovars were Grippotyphosa, Autumnalis and Sejroe with a seropositivity of 19.01 per cent. Soman *et al*. (2014) observed Hardjo as the predominant serovar whereas Canicola was not detected in any of the samples from apparently healthy cattle in Thrissur which is in agreement with the present study. The higher seroprevalence in the study may be because the samples were collected during post flood period when the environmental conditions were favourable for the survival and propagation of the organism. Vegad and Katiyar (2001) mentioned that infected cattle can void leptospires in their urine for a period ranging between 10-118 days. Thus, the higher prevalence of anti-leptospiral antibodies in cattle in the present study indicated greater possibility of transmission of the disease by these animals mainly through excretion in urine. This can contaminate the environment and transmit the infection to other animals and humans. The presence of water logged areas, rodent infestation in the vicinity and environmental temperature also would

have favoured the perpetuation of leptospires in the environment.

Conclusion

The higher seroprevalence of leptospirosis in the present study indicates endemicity of the disease in the area and the role played by apparently healthy cattle in the maintenance and transmission of the disease. A comprehensive approach to understand the epidemiology of leptospirosis needs to be undertaken preferably through a 'One Health' approach to know the role of cattle, human and environment in the disease transmission for the effective implementation of preventive strategies against leptospirosis.

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Antibiogram of *Escherichia coli* isolates from faecal samples of neonatal calf diarrhoea in Wayanad district*

M. Xavier[1], A. Janus[2], P. M. Deepa[3], K. C. Bipin[4], B. P. Habeeb[5], K. Vijayakumar[6]
Department of Veterinary Epidemiology and Preventive Medicine, College of Veterinary and Animal Sciences, Pookode, Kerala Veterinary and Animal Sciences University.

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Abstract

Thirty faecal samples were collected from diarrhoeic neonatal calves reared in organized farms and households of Wayanad district. The collected rectal swabs were subjected to bacterial isolation and biochemical tests. Based on their cultural and biochemical characteristics isolates were confirmed as Escherichia coli. Based on clinical signs and bacterial isolation calves were diagnosed as affected with colibacillosis. Antibiotic sensitivity patterns of the isolates were evaluated using standard disc diffusion method in Muller Hinton Agar. Antibiogram revealed susceptibility to Chloramphenicol (70.0 per cent), Trimethoprim/Sulphamethoxazole (60.0 per cent), Ceftriaxone (33.3 per cent), Ampicillin (33.3 per cent) and Tetracycline (3.3 per cent). The sensitivity patterns of the isolates to the three antibiotics viz. Trimethoprim/Sulphamethoxazole, Ceftriaxone and Ampicillin correlated well with clinical response.

Keywords: *Escherichia coli, Antibiogram, Wayanad,*

Among the economically important diseases of bovine calves, neonatal diarrhoea caused by *Escherichia coli*, is very important and is associated with morbidity, retarded growth and mortality. Incidence of colibacillosis were reported from different states of India including Kerala. Amritha *et al*., (2018) detected pathogenic *Escherichia coli* strains from diarrheic calves of Thrissur district.

Control of this disease requires antibiotic therapy along with supportive therapy. Selection of antibiotics for colibacillosis is very important as antibiotic resistance to this pathogen has been reported by many scientists (Malik *et al.,* 2013; Moreno *et al*., 2006). Hence this study was undertaken with the objective of analysis of antibiogram and detection of suitable antibiotics against *Escherichia coli* isolates from neonatal calves.

**Forms part of the MVSc thesis submitted by the first author to the Kerala Veterinary and Animal Sciences University, Pookode, Wayanad, Kerala.*

1. *MVSc scholar and corresponding author (Email : drxaviervet@gmail.com)*

2,3,4. Assistant Professors

5. *Assistant Professor, Department of Clinical Medicine*

6. *Professor and Head, Department of Veterinary Epidemiology and Preventive Medicine, CVAS, Mannuthy*

Materials and methods

Thirty calves with neonatal diarrhoea from various parts of Wayanad district were selected for the study.

Faecal samples were collected directly from the rectum of diarrhoeic calves in a sterile collection bottle for isolation and identification of *Escherichia coli* and subsequent antibiogram studies.

For isolation of *Escherichia coli* from faecal samples standard protocol by Quinn *et al*. (2011) was followed. All the samples were first inoculated on MacConkey's agar and the gram-negative lactose fermenting bacilli obtained were further plated into Eosin Methylene Blue media. Colonies with typical metallic sheen were preserved in Brain Heart Infusion (BHI) slants for biochemical tests *viz.* Indole test, Methyl Red test, Voges–Proskauer test, Citrate test, Urease Test and Motility test. Based on the cultural, morphological, staining characteristics and biochemical reactions the isolates were confirmed as *Escherichia coli.* Based on clinical signs and results of biochemical characterization the condition was diagnosed as colibacillosis.

In-vitro antibiotic sensitivity of isolates was studied using disc diffusion technique (Bauer *et al*., 1966). A loopful of *Escherichia coli* inoculum was applied on the surface of a Mueller-Hinton agar plate using a sterile cotton swab and the plate was kept covered for 15 minutes at room temperature for drying the inoculum. The antibiotic discs were then placed 20 mm apart and they were gently pressed on to the surface of the agar to ensure contact. Plates were then incubated at 37°C for 18 to 24hrs.

Himedia antibiotic discs Ampicillin (10 mcg), Ceftriaxone (30 mcg), Trimethoprim/ Sulphamethoxazole (1.25mcg / 23.75mcg), Chloramphenicol (10mcg) and Tetracycline (30mcg) were used in this study

Results and discussion

Antibiogram revealed susceptibility to Chloramphenicol (70.0 per cent), Trimethoprim / Sulphamethoxazole (60.0 per cent), Ceftriaxone (33.3 per cent), Ampicillin (33.3 per cent) and Tetracycline (3.3 per cent).

Resistance levels were Tetracycline (83.3 per cent), Ceftriaxone (50.0 per cent), Ampicillin (30.0 per cent) Trimethoprim/ Sulphamethoxazole (16.7 per cent) and Chloramphenicol (6.7 per cent).

The antibiotic therapy was initiated with Sulphadiazine trimethoprim and with response and antibiogram results antibiotic was changed if necessary.

The sensitivity patterns of the isolates correlated well with clinical response. The antibiogram of the isolates is represented in Table 1.

Moreno *et al*. (2006) found out susceptibility of *Escherichia coli* against Trimethoprim/Sulphamethoxazole (70.7 per cent) which is well correlated with the present study in which results revealed a 60 per cent of Trimethoprime / Sulphamethoxazole.

Zhang *et al.* (2012) reported 89.69

Table 1. Antibiogram of *Escherichia coli* isolates.

Antibiotic used	Sensitive		Intermediate sensitive		Resistant	
	No. of samples	Per cent	No. of samples	Per cent	No. of samples	Per cent
Chloramphenicol	21	70.0	7	23.3	2	6.7
Trimethoprim/ Sulphamethoxazole	18	60.0	7	23.3	5	16.7
Ceftriaxone	10	33.3	5	16.7	15	50.0
Ampicillin	10	33.3	11	36.7	9	30.0
Tetracycline	1	3.3	4	13.3	25	83.3

per cent resistance against tetracycline. This resistance is highly correlating with *Escherichia coli* (83.3 per cent resistance) isolates of Wayanad district.

White *et al.* (2000) reported 90 per cent of *Escherichia coli* isolates from Georgia were resistant against Chloramphenicol and that *flo* gene was responsible for the resistance. But in this study 70 per cent of *Escherichia coli* isolates were sensitive to Chloramphenicol and absence of *flo* gene in these isolates maybe the reason for sensitivity.

Li *et al.* (2017) reported 7.3 per cent resistance to Ceftriaxone against *Escherichia coli* from China which is not in accordance with the current study. In the present study, 50 per cent of the *Escherichia coli* isolates showed resistance to Ceftriaxone.

Mohammed *et al.* (2019) reported a marked resistance of *Escherichia coli* against Ampicillin, *i.e.* 100 per cent from Egypt. But in the present study, 33.3 per cent of the *Escherichia coli* isolates from Wayanad, showed sensitivity to Ampicillin.

Even though dendrogram analysis (Fig. 1) revealed the close relation of action between Trimethoprim/Sulphamethoxazole and Chloramphenicol, i.e. variance between these two antibiotics were below 10 per cent, the current study did not use chloramphenicol in treatment as it cannot be used in food

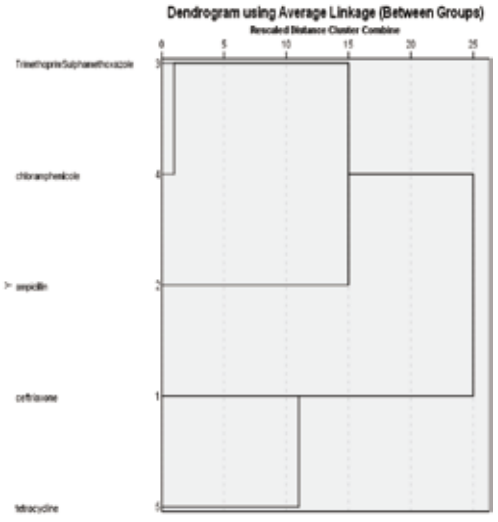
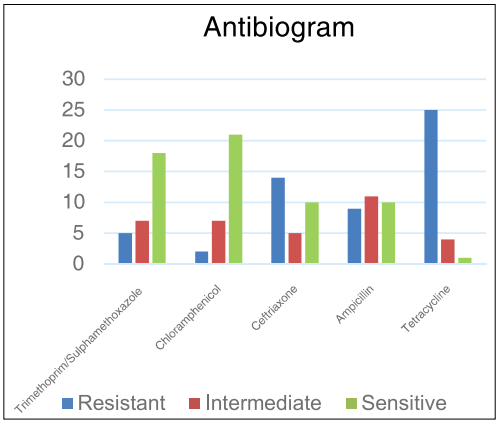


Fig 1. Dendrogram comparison of antibiogram results.



animals. But as per current antibiogram result Chloramphenicol can be used in MDR *Escherichia coli* in human medicine.

To conclude, *Escherichia coli* isolated *from* Wayanad district, exhibited much sensitivity towards Trimethoprim/Sulphamethoxazole and Chloramphenicol and high resistance towards Ampicillin and Ceftriaxone.

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RESEARCH ARTICLE
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Constraints in participatory management perceived by the inhabitants of human-wildlife conflict (HWC) affected areas in Kerala*

P. Vinoth[1], T.S. Rajeev[2], R.S.Jiji[3], R. Senthilkumar[4], V.L. Gleeja[5]
Department of Animal Husbandry Extension College of Veterinary and Animal Sciences, Mannuthy, Thirssur. Kerala Veterinary and Animal Sciences University.

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Abstract

Human-Wildlife Conflict (HWC) can be explained as an interaction between humans and wildlife where negative consequences, whether perceived, exists for one or both the parties when the action of one has an adverse effect on the other. The human population residing the forest buffer zones sharing a common geographical border with it are considered to be more affected with HWC. These populations encompassing the tribal and non-tribal communities usually manage the HWC by external assistance and internal efforts by the affected one. The present study was conducted in such a community so as to identify the above type of affect to identify the constraints in management of HWC encountered by tribal and non-tribal the inhabitants associated with the Eastern Palakkad forest circle of Kerala state in India. A total of sixty respondents including thirty non-tribal and tribal families each from the affected people were purposively selected for study various division of Palakkad forest area. The responses were recorded initially with group discussion, pilot study etc., and the final schedule was used as the scale with final statement to analyse the constraints of the respondents using Garret ranking method. The constraints faced by encountering HWC and implementing control measure. Most of the non-tribal and tribal respondents perceived HWCs to be caused by water scarcity during the summer period and inadequate food availability due to climate change and deforestation, shrinkage of grassland/pasture land, lack of proper barriers (solar fence, trench) and proximity to forest land as the major constraints.

Keywords: *Constraints, Human-wildlife conflict, Conservation, Participatory management*

Kerala, in the south-western state of India, possesses a forest area of 11309.50 Sq.km which occupies 29.1 per cent of its land area. The participatory management of HWC involves a

**Forms part of the MVSc thesis submitted by the first author to the Kerala Veterinary and Animal Sciences University, Pookode, Wayanad, Kerala.*

1. *MVSc scholar and corresponding author (Email : vinoth30894@gmail.com)*
2. *Assistant Professor*
3. *Professor and Head*
4. *Assistant Professor*
5. *Assistant Professor, Department of Statistics*

common outlook with diversities in objectives and entrust the affected folk with the decision-making process and governance. It accelerates conservation by allowing local people to participate in natural resources management and empowers them through sustainable use of forest produces and benefit-sharing. It also leads to poverty alleviation and sustainable economic development of the human population associated with wildlife habitat in the buffer zone (Scherl *et al*., 2004; Balint, 2006). In India, studies of India's Joint-Forest Management (JFM) programs have shown that despite attempts to reduce marginalization of people along lines of caste, ethnicity, and gender, the participation of people in these groups remains limited in many cases (eg., Sarin 1998; c.f., Agarwal 2000; Menzies 2003). Rohini *et al.* (2016) reported a total of 277 incidents of crop depredation, 12 incidents of property damage, three human injuries and one human death due to conflict from June 2014 to May 2015 in Nilambur, Western Ghats of Kerala. Such kind of heavy impact conflicts lead to an impairment of various measures for the conservation of wildlife which in turn affect the existence of a diverse range of wildlife and resources of the state. Several methods have been taken up successfully by stakeholders. These include methods that are direct, indirect and participatory in nature. In Bolivia, communities were involved in *chaku* (wildlife drives), a multimodal repellent procedure in which large numbers of community members move through grazing areas making noise, holding lit firecrackers, and generally clearing the way of predators and grazing competitors (Treves *et al.,* 2009).

HWC could be generally explained as the interaction between humans and wildlife where negative consequences, whether perceived, exists for one or both the parties when the action of one has an adverse effect of the other party (Conover, 2001). Management of human-wildlife conflict is one of the important challenges to the wildlife researchers, conservationists and forest managers. The major reason for human-wildlife conflict could be due to the invasion of agriculture fields on the forest fringe areas and various developmental activities in the forest region. Fragmentation of habitats evading to trapping of elephants in isolated patches with cultivation all around is mentioned as the a factor responsible for crop-raiding in South India. Further, factors such as degradation of habitat, competition for water, movement pattern, palatability and nutritive value of crops also lead to crop depredation. The Eastern Forest circle, Kerala has a sizeable wildlife population and viable habitat. The term conservation refers to the protection, care, management and maintenance of ecosystems, habitats, wildlife species and populations, within or outside of their natural environments, in order to safeguard the natural conditions for their long-term permanence (IUCN, 1980). An in-depth investigation of the views, beliefs, perceptions and constraints in HWC and wildlife conservation are essential for introducing any scientific intervention for further improvement in the existing management system so as to prevent conflicts. Hence, the present study was carried out to identify the constraints in HWC.

Materials and Methods

A total of sixty respondents including thirty non-tribal and tribal families each from the affected folk were purposively selected for study from various divisions of Palakkad forest area. The list of non-tribal and tribal families directly involved or affected by the attacks by wild animals from each division was prepared from the available records of the Kerala Forest and Wildlife Department was prepared. A total of twelve people from each division, six tribal and six non-tribal, were purposively selected from the list of affected or people from each division. The responses were recorded initially with group discussion, pilot study *etc*., and the final schedule was used as a scale with final statements to analyse the constraints of the respondents using Garret ranking method. The constraints faced by the tribal and non-tribal families encountering human wildlife conflict are discussed below.

Results and Discussion

Data presented in Table 1 revealed that 90 per cent of non-tribal respondents perceived both water scarcity during the summer period and inadequate food availability due to climate change and deforestation as the

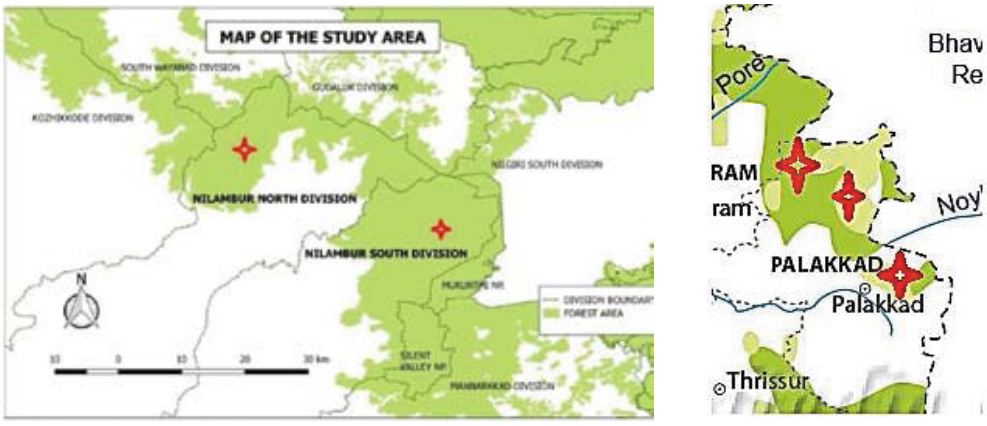

Fig. 1. Map showing the location of the study

major constraints. Shrinkage of grassland/ pasture land and lack of proper barriers (solar fence, trench) were perceived as next major constraint by 83.33 per cent and 73.33 per cent of respondents respectively. Other constraints perceived by the respondents included proximity to forest land (66.67 %), encroachment to the forest area (60 %), migration of settlements to deep forest areas (53.33 %), recommended technologies are costlier for management of wildlife conflicts (46.67 %) and lack of awareness and training of stakeholders (40 %).

Data presented in Table 1 revealed that majority of the tribal respondents (96.67 per cent) perceived water scarcity during the summer to be a major constraint. Other constraints are inadequate food availability due to climate change and deforestation (86.67 per cent), shrinkage of grassland/pasture land (73.33 per cent), lack of proper barriers (solar fence, trench) (66.67 per cent), encroachment to the forest area (63.33 per cent), inadequate financial support (56.67 per cent) and lack of awareness and training of stakeholders (43.33 per cent).

Table 1. Distribution of the non-tribal and tribal respondents based on the HWC constraints perceived by them

S. No.	Statement	Non-tribal		Tribal	
		Percentage (%)	Rank	Percentage (%)	Rank
1	Water scarcity during the summer period	90	I	96.67	I
2	Inadequate food availability due to climate change and deforestation	90	I	86.67	II
3	Shrinkage of grassland/pasture land	83.33	II	73.33	III
4	Lack of proper barriers (solar fence, trench)	73.33	III	66.67	IV
5	Proximity to forest land	66.67	IV		
6	Encroachment to the forest area	60	V	63.33	V
7	Migration of settlements to deep forest areas	53.33	VI		
8	Recommended technologies are costlier for management of wildlife conflicts	46.67	VII		
9	Inadequate financial support			56.67	VI
10	Lack of awareness and training of stakeholders	40	VII	43.33	VII

Similar report by Ramkumar *et al.* (2014) pointed out the major reasons for human-elephant conflict as non-availability of food plants in forest, water scarcity (29.2 per cent, increase of elephant population (15.5 per cent) and elephant's preference for agricultural crop (13.9 per cent) over forest plants.

Conclusion

Most of the non-tribal respondents perceived water scarcity during the summer period, inadequate food availability due to climate change and deforestation as the major constraints in controlling HWC. The shrinkage of grassland/pasture land, lack of proper barriers (solar fence, trench), proximity to forest land, encroachment to the forest area, migration and encroachment human settlements to deep forest areas, costlier technologies in managing HWC introduced and lack of awareness and training of stakeholders were the other perceived constraints. Almost all the tribal respondents perceived that water scarcity during the summer period as the major constraint followed by inadequate food for animals inside forest, deforestation, shrinkage of grassland/pasture land, lack of proper barriers (solar fence, trench), encroachment to the forest area, inadequate financial support to control HWC and lack of awareness and training to the stakeholders as other constraints in that order. It is worth to note that the tribal people did not feel the proximity to forest to be a constraint.

Most of the non-tribal respondents perceived that lack of awareness about conservation of wildlife, lack of training, inadequate financial support and costlier recommended technologies in management etc., to be the major constraints, whereas most of the tribal respondents perceived lack of awareness in conservation of wildlife, inadequate financial support for conservation, costlier recommended technologies etc., as major constraints.

The above findings regarding the constraints perceived by tribal and non-tribal families in the management of HWC throws light on the need for modification of policy and programme to address the issues as perceived by the affected people. The above findings are supported by Ramkumar *et al.* (2014) who pointed out that non-availability of food plants in the inside the forest, water scarcity, increase in elephant population and elephant's preference to agricultural crop over forest plants as food as the major constraints in controlling human wildlife conflict. With the information collected from the respondents of study area it can be concluded that, strict measures and proper management facilities should be provided for effectively minimising HWC.

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RESEARCH ARTICLE
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Assessment of foetal lung surfactant in amniotic fluid of dogs by bubble test, to evaluate foetal maturity*

D.S. Suprith[1], M.P. Unnikrishnan[2], M.O. Kurien[3], B. Bibin Becha[4] and Binu K Mani[5]
Department of Animal Reproduction, Gynaecology and Obstetrics, College of Veterinary and Animal Sciences, Mannuthy, Thrissur- 680 651. Kerala Veterinary and Animal Sciences University, Pookode, Wayanad

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Abstract

Study was conducted to determine the reliability of bubble test for assessment of lung surfactant in amniotic fluid and its application in clinical level to judge the foetal maturity. Amniotic fluid samples were collected from the foetuses of dogs undergoing elective caesarean section (Group I) at term and from the foetuses of dogs undergoing ovariohysterectomy at different stages of gestation, following misalliance (Group II). All the samples from neonates of Group I were found positive for bubble test, with variable grades ranging from two to four. All the samples from Group II foetuses were found negative for bubble test, with grade of zero and one. The results signify the presence of adequate foetal surfactant in amniotic fluid of mature foetuses when compared to that of immature foetuses and also suggest the potential practical utility of bubble test for foetal maturity assessment, particularly for timing elective induction of whelping or timing of caesarean delivery.

Keyword: *Bubble test, Foetal maturity, Amniotic fluid, Surfactant, Gestation*

Although 60 to 80 per cent of pregnant dogs suffering from dystocia end up in emergency caesarean section (Bergstrom *et al*., 2006), risk to the dam and neonate is much higher (Moon *et al.,* 2000). Elective caesarean section (C-section) is an alternate approach but, with the risk of delivery of premature foetuses.

Rise in foetal adrenocorticosteroids and the decrease of progesterone concentration

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1. *MVSc Scholar, Dept. of Animal Reproduction, Gynaecology and Obstetrics, Email id: suprithraj4@gmail.com*
2. *Assistant Professor, Centre for Pig Production and Research, Mannuthy, Email id: unnikrishnan@kvasu.ac.in*
3. *Professor and Head (Retd.), Dept. of Animal Reproduction, Gynaecology and Obstetrics, CVAS, Mannuthy*
4. *Assistant Professor, Livestock Research Station, Thiruvizhamkunnu, Palakkad*
5. *Assistant Professor, Department of Veterinary Microbiology, CVAS, Pookode*

during the final period of pregnancy are associated with the maturation of vital organs, including lung (Bonte *et al.,* 2017). Production of lung surfactant is the critical phase in the progression to lung maturity. It prevents atelectasis in neonates, by lowering alveolar surface tension, through formation of a phospholipid-rich monolayer between gas and liquid surfaces of alveolar epithelial cells. Impairment in the production of surfactants causes most significant pathological condition called respiratory distress syndrome (Vannucchi *et al.,* 2012).

Surfactant is produced by Type II pneumocytes during the saccular stage of lung development; *in-utero* foetal breathing movements and ciliated epithelium of the respiratory passages promotes diffusion of alveolar surfactant into amniotic fluid (Castagnetti *et al.*, 2007). This leads to the concept of sampling amniotic fluid for assessing foetal lung maturity. However studies for assessing foetal lung surfactant in dogs are lacking. Hence, the study was conducted to assess the presence of lung surfactant by bubble test, in the amniotic fluids collected during two different stages of pregnancy among dogs.

Materials and methods

The present investigation was carried out in University Veterinary Hospital, Kokkalai. A total of 12 dogs belonging to five different breeds were selected for the study and was divided in to two groups (Group I and II) of six each. Group I consisted of dogs which were undergoing elective C-section, when the serum progesterone level was < 2 ng/mL and Group II consisted of dogs which were undergoing ovariohysterectomy between 30 to 45 days of gestation following misalliance. About 1.5 to 3 mL each of amniotic fluid sample was aspirated from every foetal bag, into a 5 mL syringe, by needle puncture of amniotic sac between the foetal legs, before opening the foetal bags.

Amniotic fluid samples were analysed for the presence of lung surfactant, using bubble test as described by Bonte *et al.* (2017). Briefly, 1 mL of amniotic fluid sample was pipetted into a clean glass test tube and about 1 mL of 95 per cent of ethanol was added to it. Test tube was shaken vigorously for 30 sec and allowed to stand for 15 to 30 sec at room temperature and examined for the presence of bubbles at the meniscus. Grading of results was done by counting the number of layers of stable bubbles and inferred as depicted in Table 1.

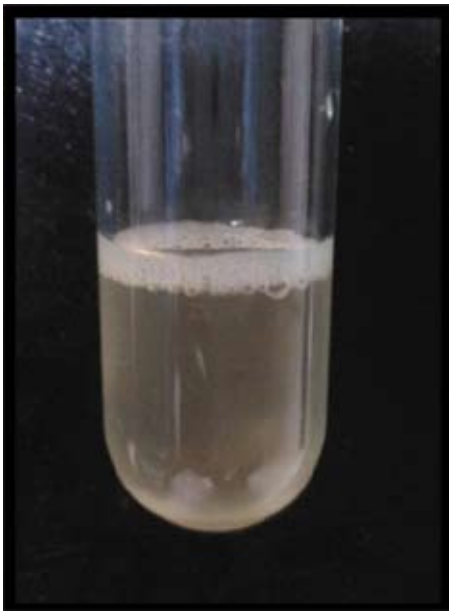
Table 1. Bubble test grading system used in the study

Observation	Grades	Inference
No bubbles	0	Negative
A single layer of bubbles	1+	
Two layers of bubbles	2+	Positive (Sufficient surfactant for *extra-uterine* life)
Three layers of bubbles	3+	
Whole surface of the tube covered with bubbles	4+	

Table 2. Number and per cent of amniotic fluid samples showing different grades during bubble test

Group	No. of samples	Number and per cent of amniotic fluid samples with different bubble test grades				
		Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
I	24	0	0	11 (45.83%)	9 (37.5%)	4 (16.67%)
II	27	18 (66.67 %)	9 (33.33%)	0	0	0

Pictures showing positive and negative bubble test results in amniotic fluid



Positive bubble test result

Negative bubble test result

Results and Discussion

A total of 51 amniotic fluid samples were collected, among which 24 from Group I and 27 from Group II dogs.

All amniotic fluid samples of Group I were found positive for bubble test with variable grades ranging from two to four whereas all the samples from Group II were found negative for bubble test, with grade of zero and one. In Group I, 45.83, 37.5 and 16.67 per cent of samples showed bubble score of two, three and four, respectively. In Group II, 66.67 and 33.33 per cent of samples showed bubble score of zero and one, respectively.

The results indicate the presence of adequate lung surfactant in amniotic fluid to support *extra-uterine* life in Group I when compared to that of Group II. Jayakumar *et al.* (2018) also reported the positive results of bubble test in amniotic fluid collected from dogs undergoing C-section and negative results in amniotic fluid collected from those undergoing ovariohysterectomy at different stages of gestation. Kutzler and Volkmann (2008) observed positive result of foam stability test in amniotic fluid collected at gestational age of 61 and 62 days, as calculated from Luteinizing Hormone (LH) surge and negative results in amniotic fluid collected at 59 and 60 days, past LH surge.

Negative results in amniotic fluids collected during ovariohysterectomy at around 30 to 45 days of gestation in Group II foetuses could be attributed to fact that in canines, saccular stage of lung development was observed around 57 and 60 days of pregnancy and surfactant production might occur during that phase only (Sipriani *et al.,* 2009).

The results signify the presence of adequate foetal surfactant in amniotic fluid of mature foetuses when compared to that of immature ones and also suggest the potential practical utility of bubble test for foetal maturity assessment, particularly for timing elective induction of whelping or timing of elective C-section. Further studies are also essential to develop a harmless technique of collecting amniotic fluid from foetuses *in-utero*, like trans-abdominal amniocentesis and studies in large number of mature and premature puppies, which can find practical application in the management of canine pregnancy.

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RESEARCH ARTICLE
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Occurrence of repeat breeding and prolonged oestrus in crossbred cattle*

H.D. Arun[1], B. Bibin Becha[2], C. Jayakumar[3], M.P. Unnikrishnan[4], K.S. Ajith[5] and M.O. Kurien[6]
Livestock Research Station, Thiruvazhamkunnu, Palakkad – 678 601
Kerala Veterinary and Animal Sciences University, Pookode.

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Abstract

Study was conducted to determine the occurrence of repeat breeding and prolonged oestrus among crossbred cattle in organized farms. Data were collected from breeding registers maintained on farms over a period of four years and tabulated in relation to age and parity. Overall occurrence of repeat breeding, prolonged oestrus and occurrence of prolonged oestrus among repeat breeder animals was 25.96 per cent, 25.86 per cent and 55.42 per cent, respectively. Occurrence of repeat breeding, prolonged oestrus and repeat breeding among prolonged oestrus exhibiting animals among cows was 27.35, 30.29, and 61.58 per cent, respectively. Occurrence of repeat breeding, prolonged oestrus and repeat breeding among animals exhibiting prolonged oestrus, among heifers was 23.07, 16.66, and 40.27 per cent, respectively. Among cows, the occurrence of repeat breeding was highest in the age group of 2-4 years and occurrence of prolonged oestrus among repeat breeders was highest in the age group 8-12 years.

Keywords: *Repeat breeding, prolonged oestrus, crossbred cattle*

Repeat breeding is one of the major problems in dairy industry and accounts for substantial economic losses. Repeat breeding in cattle (RB) is defined as a failure of animals to conceive from three or more regularly spaced services in the absence of detectable abnormalities (Mesafint and Guesh, 2014). Prolonged oestrus is defined as lengthened duration of oestrus in

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1. *MVSc Scholar, Dept. of Animal Reproduction, Gynaecology and Obstetrics, CVAS, Mannuthy E-mail: arunhd25@gmail.com*
2. *Assistant Professor, Livestock Research Station, Thiruvazhamkunnu, Palakkad, E-mail: bibinbecha@kvasu.ac.in*
3. *Assistant Professor and Head (i/c), Dept. of Animal Reproduction, Gynaecology and Obstetrics, CVAS, Mannuthy*
4. *Assistant Professor, Centre for Pig Production and Research, Mannuthy*
5. *Assistant Professor, Department of Animal Nutrition, CVAS, Mannuthy*
6. *Professor and Head (Rtd.), Dept of ARGO, CVAS, Mannuthy*

cattle and it is one of the major contributors to repeat breeding (30-40 percent) in crossbred cattle. Prolonged oestrus leads to delayed ovulation which results in asynchrony between ovulation and insemination which in turn results in fertilization failure (Bage *et al*., 2002). This retrospective study was conducted to determine the occurrence of repeat breeding, prolonged oestrus and prolonged oestrus with repeat breeding as well as the effect of age and parity on these conditions.

Materials and Methods

Data for a period of four years was collected from the breeding registers maintained at Livestock Research Station (LRS), Thiruvazhamkunnu and University Livestock Farm and Fodder Research and Development Scheme (ULF and FRDS), Mannuthy from January 2015 to December 2018.

A cow was considered to be a repeat breeder (RB) if it was diagnosed as non pregnant, even after three or more consecutive artificial inseminations. Cows with conditions like cystic ovaries, anoestrus, endometritis *etc.* were excluded from the study. Animals that exhibited oestrus for a duration of more than one day (24 hours) were considered as animals with condition prolonged oestrus. Data collected were tabulated according to different age groups (2-4 years, 4-8 years and 8-12 years) and parity (0, 1, 2, 3, 4 and above). Data were analyzed for occurrence of repeat breeding, prolonged oestrus and occurrence of prolonged oestrus among repeat breeding animals.

Results and Discussion

Overall occurrence of repeat breeding, prolonged oestrus and prolonged oestrus

Table 1. Overall occurrence of repeat breeding, prolonged oestrus and prolonged oestrus among repeat breeding crossbred cattle

Animals	Repeat breeder animals	Animals exhibiting prolonged oestrus	Repeat breeders exhibiting prolonged oestrus
Cow	177 (27.35 %)	196 (30.29 %)	109 (61.58 %)
Heifer	72 (23.07 %)	52 (16.66 %)	29 (40.27 %)
Total	**249 (25.96 %)**	**248 (25.86 %)**	**138 (55.42 %)**

Table 2. Occurrence of repeat breeding, prolonged oestrus and prolonged oestrus among repeat breeding animals of different age group

Age group	Repeat breeder animals	Animals exhibiting prolonged oestrus	Repeat breeders exhibiting prolonged oestrus
2-4 years (n=388)	107 (27.57 %)	111 (28.60 %)	58 (54.20 %)
5-8 years (n=453)	110 (24.28 %)	103 (22.73 %)	60 (54.54 %)
8-12 years (n=148)	34 (22.97 %)	34 (22.97 %)	26 (76.47 %)

Table 3. Occurrence of repeat breeding, prolonged oestrus and prolonged oestrus animals among repeat breeders of different parity

Parity	Repeat breeder animals	Animals exhibiting prolonged oestrus	Repeat breeders exhibiting prolonged oestrus
0 (n=312)	70 (22.41 %)	58 (18.58 %)	33 (47.62 %)
1 (n=164)	48 (32.32 %)	48 (29.26 %)	27 (50.94 %)
2 (n=204)	47 (23.03 %)	54 (26.47 %)	28 (59.27 %)
3 (n= 133)	32 (24.06 %)	38 (28.57 %)	21 (65.62 %)
4 and above (n= 156)	49 (31.41 %)	57 (36.53 %)	40 (81.63 %)

among repeat breeder cattle and the occurrence in cows and heifers are shown in Table 1. Overall occurrence of repeat breeding in crossbred cattle was found to be 25.96 per cent. The results are in agreement with Nanda and Singh (2008) who reported an occurrence of 20 to 30 per cent repeat breeding. The reason for the higher occurrence of repeat breeding among cows when compared to heifers in present study may be due to factors like negative energy balance after calving, parturient and post parturient complications, lactational stress *etc* (Mesafint and Guesh. 2014). Overall occurrence of prolonged oestrus was 25.86 per cent which is in agreement with Shakir (2018) who reported that prolonged oestrus had an occurrence of 26.87 per cent prolonged oestrus. Among 249 repeat breeders, 138 animals exhibited prolonged oestrus with an occurrence of 55.42 per cent which is in agreement with Cummins *et al*. (2012). Elevated plasma progesterone level at oestrus decreases the preovulatory LH surge resulting in an extension of duration of oestrus duration leading to asynchrony between ovulation and insemination resulting in fertilization failure thus contributing to repeat breeding (Singh *et al.,* 2012).

Occurrence of repeat breeding, prolonged oestrus and prolonged oestrus among repeat breeder cattle in relation to age is shown in Table 2. Occurrence of repeat breeding was found to be higher in the age group of 2-4 years. This may be attributed to the negative energy balance and stress due to lactation. Animals that exhibited prolonged oestrus among the repeat breeders were highest in the age group of 8-12 years which can be attributed to the endocrine disturbances occur along as age increases. Negative energy balance and stress due to production alters insulin metabolism and leads to decreased sensitivity of corpus luteum to endogenous prostaglandins and which was also required for normal development of follicles (Singh *et al.,*2012).

Occurrence of repeat breeding, prolonged oestrus and prolonged oestrus among repeat breeder cattle in relation to parity is shown in Table 3. Parity wise analysis of data showed that repeat breeding was highest among animals that had calved only once and this may be due to lactational stress, negative energy balance, impairment of follicular development resulting in fertilization failure and early embryonic death (Mihm *et al*., 1999). Occurrence of prolonged oestrus was highest among repeat breeders having parity four and above, and this may be due to endocrine disturbances and anatomical defects acquired as parity increases (Asaduzzaman e*t al*., 2016 and Safna, 2007).

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Clinicopathological profile of *Babesia canis vogeli* infection in dogs*

Ancy Thankachan[1], K. Vinodkumar[2], Shyma V.H[3], R. Asha[4], K. Vijayakumar[5]
Department of Veterinary Epidemiology and Preventive Medicine College of Veterinary and Animal Sciences, Mannuthy 680651, Thrissur, Kerala.
Kerala Veterinary and Animal Sciences University

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Abstract

The clinicopathological profile of Babesia canis infection in dogs presented to University Veterinary hospital, Kokkalai was studied. Eight animals with clinical signs of babesiosis like pyrexia, anorexia, amaemia and lethargy and found positive for Babesia piroplasms in blood smear were included in the study. The organism was identified as Babesia canis vogeli by PCR. Haematological analysis showed highly significant alterations in granulocyte count, monocyte count, Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), platelet count and significant alterations in total RBC count. Systemic inflammatory response syndrome (SIRS), as per the classification of Matijatko *et al*. (2010) *was noticed in two out of eight animals. This indicate that B. c. vogeli organisms which are considered to be benign in some parts of the world can cause severe complications in a different geographical location.*

Keywords: *Babesiosis, dogs,* SIRS

Canine babesiosis is considered as the most important haemoprotozoan disease of pet dogs all over the world. The haemoprotozoan *Babesia canis* is a common piroplasm affecting the erythrocytes of the dogs of India and is transmitted by the brown dog tick *Rhipicephalus sanguineus* (Dantas- Torres, 2010). There are mainly three genotytpes viz. *Babesia canis canis, Babesia canis rossi* and *Babesia canis vogeli* (Uilenberg, 2006; Irwin, 2009; Eichenberger *et al.,*2016), among which, *B. c. vogeli* is reported to cause only clinically unapparent infections. Anorexia, lethargy, dyspnoea and haemoglobinuria are the most common clinical signs noticed. Animals that are positive for *Babesia canis* piroplasms and having severe clinical signs are occasionally presented

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1. *MVSc Scholar, email id: ancythankachan2011@gmail.com*
2. *Assistant Professor, email id: vinodkumar@kvasu.ac.in*
3. *Assistant Professor, email id: shyma@kvasu.ac.in*
4. *Assistant Professor, Department of Veterinary Parasitology, email id: asha@kvasu.ac.in*
5. *Professor and Head, email id: vijayakumar@kvasu.ac.in*

to the veterinary hospitals in Kerala. So this study was conducted to identify the genotype of babesia causing this severe form of disease in our state.

Materials and Methods

Animals

Eight animals that were presented to University Veterinary Hospital Kokkalai with severe clinical signs of babesiosis like pyrexia, anorexia, lethargy and dyspnoea, and found positive for *B. canis* piroplasms in blood smears under Field's staining were included in the study. Detailed clinical examination of the animals was done.

Preliminary examination

General examination, physical examination and clinical examination of all animals were done and results recorded. Peripheral blood smears from the tip of ears were collected,stained using Field staining and examined under oil immersion objective of microscope (100x).

Haematological analysis

Two ml of blood was collected from the saphenous or medial cephalic vein of each dog under aseptic techniques. The blood was transferred to a collection tube with EDTA (Hi Media, Mumbai). Complete blood count included the following parameters; Total erythrocyte count (TEC) (10^6/µl), Total leukocyte count(TLC) (10^3/µl),differential leukocyte count (%), Mean corpuscular volume (MCV) (µm^3), Mean corpuscular haemoglobin (MCH) (pg), Mean corpuscular haemoglobin concentration (MCHC (g/dl) and Thrombocyte count (10^3/ µl) were analysed using Automatic Haematology Analyzer (Orphee, Mythic Vet 18) was used.

Molecular identification

Extraction of DNA was done from blood using DNeasy blood and tissue kit (QIAGEN. Hilden, Germany) as per the manufacturer's protocol. The PCR reactions were performed using the MJMini™Personal Thermal cycler, and S1000 thermal cycler (Biorad, USA). The primers used were given in table 1 and followed the protocol recommended by Arthi *et al*., 2017

Analysis of PCR amplicons

Products of PCR were analysed by agarose gel electrophoresis in a submarine gel electrophoresis apparatus (GeNei™, Bangalore). Subsequent to electrophoresis, the gel was transferred to a UV transilluminator (GeNeiTM) for visualization of bands. The gel was analysed in Gel Documentation System (BIO-RAD, USA).

Therapeutic management

Systemic inflammatory response syndrome (SIRS)

Classification of the affected animals into SIRS positive and SIRS negative based on the vital parameters and haematology as per Matijatko *et al*. (2010)

Two standard protocols were employed based on the severity of the clinical signs in the animals affected with canine babesiosis. Diminazineaceturate@ 3.5 – 4 mg/kgbwt intramuscularly to adult animals and pups were treated with Clindamycin, metronidazole and doxycycline at the dose rates of 11 mg/kgbwt, intravenously, 20 mg/kgbwt, intravenously, 10 mg/kgbwt. once daily, per orally respectively.

Statistical analysis

The IBM - SPSS software version 24 was used to analyze numerical data gathered in the present study. The one sampled t test was choosen for analysis.

Results and Discussion

The microscopical examination of blood smear using Field's staining revealed characteristic pear shaped piroplasms of *B. canis* in pairs (Fig 1) from all the eight animals under study.

Description of animals

The age of eight animals under study ranged from 27 days to 8 years. Adaszek *et al*.

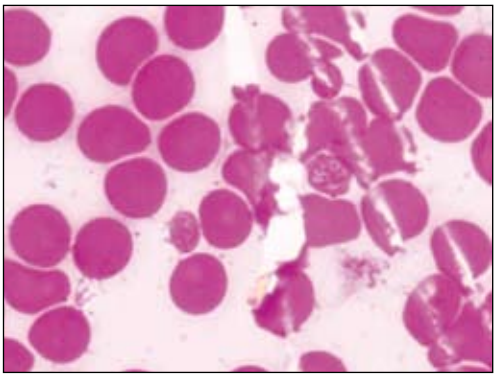

Fig 1: Pear shaped *B. canis* piroplasms **(Field staining X1000)**

(2016) reported *B. c. canis* infection in eight week old puppies. The major clinical signs noticed in eight animals understudy are given in table 1

Haemoglobinuria which was described as one of the most common clinical sign by Aleri *et al*. (2011) was noticed in three out of eight animals affected with *B. c. vogeli* under the present study. The intraerythrocytic multiplication of parasite by binary fission leads to direct erythrocyte injury and haemoglobinuria. Abortion was noticed in one dog on the 48th day of gestation, and cerebral form of babesiosis was noticed in one dog.

Haematological analysis

The statistical analysis of the haematological alterations in dogs affected with and *B. c. vogeli* are given in table3. Significant difference from standard values were noticed in total RBC, granulocyte, monocyte, MCV, MCH and platelets in the affected animals. Significant increase from standard values were noticed in granulocyte percentage, Monocyte percentage, MCV whereas significant reduction in RBC and platelets count. This was in accordance with Solano-Galego and Baneth (2011). The granulocytosis and monocytosis observed in the present study was in contrast to the findings of Schetters (2009) who has described granulocytopenia and monocytopenia.

Furlanello *et al*. (2005) has described increased consumption of thrombocytes associated with immune mediated injury, sequestration and supression of bone marrow are reasons for thromvocytopenia in dogs affected with babesiosis.

Molecular Identification

DNA extracted from the whole blood of all the affected animals was subjected to genus, species and genotype specific PCR for molecular identification of babesia organisms. The genus specific PCR for *Babesia* spp. with primer pair PIRO A and PIRO B yielded indended PCR product of approximately 400 bp, while the species specific PCR for *B. canis* with primer pair Can172F and Can626R yielded indended PCR product of approximately 454 bp and sub-species specific PCR for *B. c. vogeli* with primer pair BAB 1 and BAB 4 yielded indended PCR product of approximately 590 bp from all the eight samples. No bands were observed with negative control. The *B. c. canis* and *B. c. rossi* sub-species specific PCR with primer pair BAB 1 and BAB 3 and BAB 1 and BAB 5 did not yield amplicons from any of the samples.

Systemic inflammatory response syndrome(SIRS)

Occurrence of two or more of clinical signs like tachycardia, hypothermia, tachypnoea, hyperthermia, leukocytosis or leukopenia and neutrophilic shift was suggestive of SIRS (Matijatko *et al*., 2010). Among the eight animals affected with *B. c. vogeli* two animals were SIRS positive as per Matijatko *et al*. (2010) ,which was lower than that reported by Galan *et al*. (2018) for *B. c. rossi* and *B. c. canis* and it could be due to the lesser virulence of *B. c. vogeli*. Table 5 shows SIRS categorisation into SIRS positive and negative in selected eight animals

The two SIRS positive animals and SIRS negative adult animals were treated with Diminazine aceturate injection @ 3.5 mg/kgBwt intramuscularly once (Schoeman 2009). The SIRS negative pup was treated with the standard protocol using Clindamycin @ 11mg/kgbwt and Metronidazole @ 20mg/kgbwt intravenously, and doxycycline@10 mg/kgbwt per orally for 14 days. (Greene, C.E. 2012)

One of the SIRS positive animal was subjected to blood transfusion. But condition

Table 1. Genus, species and sub-species specific primers selected for PCR amplification of blood samples from 25 dogs. (Arthi *et al.*, 2017)

Organism	Primer name		Gene sequence 18s rRNA	Product size
Babesia genus	Forward	**PIRO A**	5'-AATACCCAATCCTGACACAGGG -3'	400bp
	Reverse	**PIRO B**	5'-TTAAATACGAATGCCCCCAAC -3'	
Babesia canis	Forward	**Can172F**	5-'GTTTATTAGTTTGAAACCCGC- 3'	454 bp
	Reverse	**Can626R**	5'-GAACTCGAAAAAGCCAAACGA- 3'	
Babesia canis vogeli	Forward	**BAB1**	5'-GTG-AAC-CTT-ATC-ACT-TAA- AGG-3'	590 bp
	Reverse	**BAB4**	5'-CAA-CTC-CTC-CAC-GCA-ATC G-3'	
Babesia canis canis	Forward	**BAB1**	5'-GTG-AAC-CTT-ATC-ACT-TAA-AGG-3'	746 bp
	Reverse	**BAB3**	5'-CTA-CAC-AGA-GCA-CAC-AGC C-3'	
Babesia canis rossi	Forward	**BAB1**	5'-GTG-AAC-CTT-ATC-ACT-TAA-AGG-3'	342 bp
	Reverse	**BAB5**	5'-AGG-AGT-TGC-TTA-CGC-ACT CA-3'	

Table 2: Clinical signs noticed for dogs affected with *B. c. vogeli*

Animal number	Anorexia	lethargy	Haemo-globinuria	pyrexia	Anaemia	Nasal discharge	Abortion	seizures
1	+	-	-	+	-	+	-	-
2	+	+	-	+	+	-	+	-
3	+	-	-	+	-	-	-	-
4	-	+	-	+	+	-	-	-
5	-	-	+	+	-	-	-	-
6	-	-	+	+	-	-	-	-
7	+	-	+	+	+	-	-	-
8	+	-	-	-	-	+	-	+

Table 3: General parameters of dogs affected with *B. c. vogeli*

Animal no	Temperature (°F)	Mucous membranes	Lymphnode	Heartrate (beats/min)	Respiration (breaths/min)
1	105.3	Pink	Enlarged	88	45
2	103.3	Pale	Enlarged	87	23
3	102.6	pale roseate	Enlarged	79	12
4	102.9	pale roseate	Enlarged	92	32
5	103.8	Pale	Enlarged	112	38
6	103.6	Congested	Enlarged	85	36
7	103.2	pale roseate	Enlarged	80	28
8	97	Pale	Enlarged	70	42

Table 4: Statistical analysis of haematological alterations in dogs affected with *B. c. vogeli*

Sl no	Variable	Mean±SE	Test value	t value	P value
1	Total RBC ($x10^6/mm^3$)	3.9475*± 0.60484	5.5	2.567	0.037
2	Total WBC ($x10^3/mm^3$)	6.550^{ns}± 0.5695	6	.966	0.366
3	Granulocyte (%)	71.050**± 2.9406	51.6	6.614	<0.001
4	Monocyte (%)	6.100** ± 0.7597	2.5	4.739	0.002
5	MCV (μm^3)	66.338**± 1.6391	60	3.866	0.006
6	MCH (pg)	22.713 **±0.8391	26	3.918	0.006
7	MCHC (g/dL)	34.450^{ns}± 1.7786	36	0.871	0.412
8	Platelets ($x10^3$/ µl)	58.63** ± 11.503	200	12.290	<0.001

** - Highly Significant (P < 0.01), * - Significant at (P < 0.05), ns - Non - Significant

Table 5: SIRS categorisation into sirs positive and negative in selected eight animals

Sl no	Temperature (°F)	Heartrate (beats/min)	Respiration (breaths/min)	Total WBC (x10^3/mm^3)	SIRS Positive or Negative
1	105.3	88	45	**7**	Negative
2	103.3*	87	23	4*	Positive
3	102.6	79	12	6.8	Negative
4	102.9	92	32	6.1	Negative
5	103.8	112	38	9.2	Negative
6	103.6	85	36	7.5	Negative
7	103.2*	80	28	4.8*	Positive
8	97	70	42	7	Negative

D- Diminazineaceturate CMD- Clindamycin, Metronidazole and Doxycycline

Table 6: Response to therapy in dogs affected with *B. c. vogeli*

Sl no	No of animals	Clinical status	Therapeutic protocol	Progression of disease	End result
1	1	SIRS positive	D	Deterioration in body condition, complete, anorexia, lethargy, recumbency and death	Death
2	1			Gradual alleviation of clinical signs, slow restoration of appetite, complete recovery within one month	Recovery
3	5	SIRS negative Adults	D CMD	Rapid alleviation of clinical signs, regaintment of appetite, complete recovery within five days	Recovery
4	1	SIRS negative pup			

D: Diminazineaceturate, C: clindamycin, M: Metronidazole, D: Doxycycline

of animal deteriorated leading into abortion and death after two days. Table 6 shows the response to therapy in dogs affected with *B. c. vogeli.* The response to therapy was assessed with alleviation of clinical signs, becoming smear negative and with improvement in haematology.

The recovery rate in all the eight *B. c. vogeli* affected animals was 87.5 per cent and this is in agreement with Solano-Gallego and Baneth. (2011). *B. c. vogeli* is considered to be the least virulent sub species among the three causesing all the subclinical infections with low parasitemia in adult dogs. Koster *et al.* (2015a) But the findings of current study, with SIRS being noticed in two out of eight animals resulting in one fatality suggests that *B. c. vogeli* is a pathogen capable of causing severe complicated disease in affected dogs.

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RESEARCH ARTICLE
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Occurrence of vaginal hyperplasia among intact dogs*

Anila Babu[1], B. Bibin Becha[2], C. Jayakumar[3], Shibu Simon[4], Indu V. Raj[5] and M.O. Kurien[6]
Department of Animal Reproduction, Gynaecology and Obstetrics, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala – 680 651, India.
Kerala Veterinary and Animal Sciences University

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Abstract

Hyperplasia of vaginal mucous membrane is an unusual reproductive disorder observed in canines. It is the protrusion of oedematous vaginal tissue into and through the opening of the vulva occurring during proestrus and oestrus stages of the sexual cycle. This study was aimed to find out the overall occurrence of canine vaginal hyperplasia and its occurrence based on breeds, age, parity and stage of oestrous cycle. The occurrence of canine vaginal hyperplasia was 1.02 per cent. The highest occurrence of vaginal hyperplasia within a breed was observed in Labrador retrievers (48.94%), while the lowest occurrence was in German shepherd, Spitz, and Dachshund breeds (2.13%). The highest occurrence was recorded in dogs of one to two years old (43.62%) and the lowest occurrence in dogs of above 4 years of age (13.83%). Highest occurrence of vaginal hyperplasia was found in nulliparous (60.71%) and in intact dogs (100%).

Keywords: *Occurrence, Vaginal hyperplasia, Breed, Age*

Vaginal hyperplasia (vaginal prolapse, vaginal hypertrophy, oestrual hypertrophy, vaginal eversion, vaginal protrusion) is characterized by marked oedema of the sub-mucosa and stratified squamous epithelium lining the vaginal lumen, resulting in prolapse of a dome-shaped or doughnut shaped mass of tissue into the vaginal vault and through the vulvar cleft (Manothaiudom and Johnston, 1991). The condition is believed to depend on a number of factors and the exact cause is still unknown. Oestrogens are considered to play a major role in the pathogenesis of vaginal fold prolapse in the bitch because of the high incidence of the disorder during pro-oestrus,

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1. *MVSc Scholar, Dept. of Animal Reproduction, Gynaecology and Obstetrics, Email id: anilababu43@gmail. com*
2. *Assistant Professor, Livestock Research Station, Thiruvazhamkunnu, Email id:bibinbecha@gmail.com*
3. *Assistant Professor and Head (I/C)*
4. *Assistant Professor*
5. *Assistant Professor, Department of Veterinary Anatomy and Histology*
6. *Professor and Head (Retired)*

oestrus and late pregnancy, spontaneous regression of the condition in dioestrus and shrinkage and disappearance of the prolapsed mass after ovariectomy or ovariohysterectomy (Sontas *et al.,* 2010). Different treatments have been used, and some are novel approaches. Treatment depends on the extent of the vaginal fold prolapse, breeding and reproductive status. High incidence of recurrence of the condition follows medical management and hence, surgical excision of the mass is advised in breeding dogs. In dogs, not intended for breeding, prevention is accomplished if the bitch is ovariectomized.

Materials and methods

This retrospective study was undertaken to analyse the occurrence of vaginal hyperplasia in different breeds of dogs with its susceptible age, parity and stage of oestrous cycle. A review of clinical records of vaginal hyperplasia affected cases presented to the University Veterinary Hospital, Kokkalai and Mannuthy during the period of three years from June 2016 to May 2019 was utilised for this study. Occurrence of canine vaginal hyperplasia among the total number of canine gynaecological cases presented to the small animal gynaecology units of both hospitals were recorded. The cases were classified according to their breed, age, body size, and parity, stage of oestrous cycle and reproductive status of dogs.

Results and discussion

A total of 94 cases of canine vaginal hyperplasia were found among a total number of 9236 gynaecological cases presented over a period of three years, giving an overall occurrence of 1.02 per cent during the period of observation. Mcnamara *et al.* (1997) had also opined that occurrence of vaginal prolapse in female dogs is a very rare condition compared to other species such as cattle, goats and sheep.

In the present study, vaginal hyperplasia was recorded in 10 different breeds. Among different gynaecological problems, the highest occurrence of vaginal hyperplasia was observed in larger breeds like Bull Mastiff (3.60%) followed by Dobermann (2.41%) and Labrador retriever (1.84%). Among the 94 cases recorded, higher occurrence of vaginal hyperplasia was diagnosed in Labrador retriever breeds (48.94%) followed by Pug (24.47%) and Dobermann (5.32%) breeds (Table I). Similar findings were reported by Johnston (1989) and Chandrapuria and Somil (2013) who observed higher occurrence of vaginal hyperplasia in large-breed dogs like Labrador retriever, Dobermanns and among brachycephalic breeds, such as the Boxer, Bull Mastiff and Neapolitan mastiff. As against this, Kumar (2013) and Schutte (1967) reported lower occurrence of Type II and Type III vaginal hyperplasia among large breeds.

Analysis of data revealed that age wise occurrence of this condition was more in one to two years (43.62%), followed by two to three years (23.40%), three to four years (19.15%) and above four years (13.83%). The occurrence of vaginal hyperplasia was more among the dogs below two years of age (43.62%), when compared to dogs of older age groups; results were in agreement with the observations of Kumar (2013), nearly two third of the cases (63.63%) were encountered in dogs of the age group of one to two years. Many authors had reported age as a predisposing factor in vaginal fold prolapse (Schutte, 1967; Trager, 1970 and Johnston, 1989). It was noticed that occurrence of vaginal hyperplasia is more in young age groups. According to many previous reports (Trager, 1970; Manothaiudom and Johnston, 1991; Ajadi *et al.,* 2016) of the total affected dogs, vaginal hyperplasia was mostly observed in younger dogs of mean age of 18 – 22 months. On the contrary, vaginal hyperplasia in older animals especially six to seven years were reported by Post *et al.* (1991); Kim *et al.* (2008) and Jayakumar *et al.* (2016). Also, Chandrapuria and Somil (2013) reported, 60 per cent of vaginal hyperplasia affected dogs were more than five years old.

In the present study, occurrence of vaginal hyperplasia was found to be higher among large sized breeds (52%) when compared to small sized breeds (33%); results were in agreement with the observations of Manothaiudom and

Johnston (1991) in which they reported 61 per cent of the cases of Type II or Type III vaginal prolapse were seen in the dogs weighing 50 pounds or more, and only 11 per cent of the cases in dogs weighing less than 20 pounds. In contradiction to the present study, nearly ¾th of all cases of Type II or Type III vaginal prolapse recorded were in medium sized breeds comprising of Boxer and Bulldog. These observations are in agreement with the findings of Schutte (1967) and Trager (1970) who reported that Type II or Type III vaginal prolapse was observed more frequently in Boxers and Boxer crosses which come under the category of medium sized breeds.

Out of the total 94 vaginal hyperplasia cases, 61.70 per cent were belonged to nulliparous group. Screening of literature revealed similar reports that half the number of animals presented with Type II or Type III vaginal prolapse were nulliparous (Shutte, 1967 and Trager, 1970). An occurrence of 42.44 per cent vaginal hyperplasia among primiparous dogs as against 51.51 per cent in nulliparous was reported by Kumar (2013). It was observed that occurrence of vaginal hyperplasia was more among nulliparous dogs.

Among the 94 female dogs diagnosed with vaginal hyperplasia, 35.11 per cent and 60.64 per cent dogs were in proestrus and oestrus stages of ovarian cycle at the time of presentation. Similar findings were reported by the Kumar (2013) who noticed higher occurrence of vaginal hyperplasia at oestrus stage (60.60%). Various authors mentioned similar reports regarding the occurrence of vaginal prolapse during follicular stage; 81 per cent (Schutte, 1967), 73 per cent (Trager, 1970) and 86 per cent (Johnston, 1989). In the present study, a lower occurrence (4.25%) of vaginal oedema was observed during diestrus stage but, Kumar (2013) reported an occurrence of 39.39 per cent during dioestrus stage. Gouletsou *et al.* (2009) reported rare occurrence of vaginal hyperplasia in pregnancy.

In the present investigation, vaginal hyperplasia was never observed in spayed dogs. Comparable observations were reported by Kumar (2013) but an unusual case of vaginal prolapse in an ovariohysterectomised bitch was reported by Nak *et al.* (2008).

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Table 1. Breed-wise occurrence of vaginal hyperplasia (n=94)

Breed	No. of dogs diagnosed with vaginal hyperplasia	Per cent occurrence
Labrador retriever	46	48.94
Pug	23	24.47
Rottweiler	4	4.25
German shepherd	2	2.13
Dachshund	2	2.13
Spitz	2	2.13
Doberman	5	5.32
N D	2	2.13
Great Dane	2	2.13
Bull Mastiff	4	4.25
Others	2	2.13

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RESEARCH ARTICLE
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Comparison of endometrial cytology and transrectal ultrasonography for the diagnosis of subclinical endometritis*

Gayathri Prathap[1], Shibu Simon[2], M.O Kurien[3], B. Bibin Becha[5], Surej Joseph Bunglavan[4], Niyas Emadudeen[6], Gleeja V. L.[7]
Department of Animal Reproduction, Gynaecology and Obstetrics, College of Veterinary and Animal Sciences, Mannuthy, Thrissur - 680651. Kerala Veterinary and Animal Sciences University

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Abstract

The objective of the present study was to compare the efficacy of endometrial cytology (EC) and uterine echobiometry by transrectal ultrasonography (TRUS) for the diagnosis of subclinical endometritis (SCE) in crossbred dairy. A total of 20 crossbred cows, at 30 and 40 days postpartum (DPP), without any clinical signs or abnormal discharge were subjected to transrectal ultrasonography (TRUS) and endometrial cytology (EC) examination. Among the TRUS parameters, uterine horn diameter (UD) was found to be least useful in the diagnosis of SCE. Cervical diameter (CD) also had poor to moderate sensitivity when compared to EC and less accurate in diagnosing SCE. Fluid in uterus (FIU) had more sensitivity than UD and CD for the diagnosis of SCE but its presence is dependent on the stage of oestrous cycle. Hence, when TRUS is used as a supporting diagnostic aid along with EC, it has promising value in the diagnosis of SCE.

Keywords: *Subclinical endometritis (SCE), transrectal ultrasonography (TRUS), Endometrial cytology (EC).*

Successful reproduction is one of the crucial elements for a profitable dairy industry. For maintenance of high level of reproductive efficiency, healthy uterine environment, timely detection of oestrus, successful breeding and a calving interval that maximizes the milk production within the herd is required. The major economic losses in a herd attributed to infertility are reduced milk production, uterine diseases, treatment cost and increased culling rate due to failure of conception.

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1. *M.V.Sc Scholar and Corresponding author Email id: gayathriprathap31@gmail.com*
2. *Assistant Professor*
3. *Professor and Head (Rtd.)*
4. *Assistant Professor, Department of Animal Nutrition, CVAS, Pookode, Kerala, India*
5. *Assistant Professor, Livestock Research Station, Thiruvazhamkunnu, Palakkad*
6. *Research Assistant, University Livestock Farm and Fodder Research and Development Scheme, KVASU, Assistant Professor, Department of Statistics*

Postpartum period is a time when the uterine health will be compromised. It is normal to find microbial contamination of the uterus following parturition. The uterus gets contaminated with a wide range of bacterial organisms, which are normally eliminated during the process of uterine involution. However, some animals may be immunocompromised causing the persistence of the microorganisms leading to an infection. Postparturient complications like dystocia, retention of foetal membranes, uterine prolapse, metabolic diseases, nutritional and hormonal imbalance can aggravate the condition. When the postpartum uterine defence mechanisms are disrupted the endometrial lining of the uterus is affected resulting in growth of microbes and poor fertility **(**Purohit *et al*., 2015).

Endometritis can be classified as clinical (CE) and subclinical endometritis (SCE). Subclinical endometritis is difficult to diagnose as there are no visible symptoms (Foldi *et al*., 2006; Barlund *et al.,* 2008). The diagnosis of CE has been based on rectal palpation, vaginoscopy, Metricheck or ultrasonography. Diagnosis of SCE has been presumptive in the past and repeat breeder cows were considered to have SCE since transrectal palpation revealed no abnormality (Purohit, 2008). The diagnosis of SCE is based on uterine cytology where the percentage of Polymorphoneutrophils (PMN) was counted (Kasimanickam *et al.,* 2004 and Gilbert *et al.,* 2005). Brodzki *et al*. (2015) had stated that endometrial cytology could be used as an invaluable aid for diagnosing SCE in those animals without any overt manifestations. Many authors had suggested different cut off levels for PMN cells to diagnose the SCE positive animals at different days postpartum.

More recently transrectal ultrasonography (TRUS) and endometrial cytology (EC) have been suggested for the definitive diagnosis of SCE (Lenz *et al.,* 2007; Oral *et al*., 2009). Ultrasonographic features helpful in the diagnosis of SCE include accumulation of fluid, increased endometrial thickness, uterine horn diameter (UD) and cervical diameter (CD) (Lenz *et al.,* 2007; Oral *et al*., 2009; Purohit *et al*., 2015).

The presence of fluid in uterus (FIU) at three weeks postpartum in cows could be considered as a reliable indicator of endometritis, with good sensitivity and specificity (Mateus *et al.* 2002; Kasimanickam *et al.,* 2004; Barlund *et al.,* 2008 and Oral *et al.,* 2009). LeBlanc *et al*. (2002) concluded that an increase in the size of the uterus with more than 8 cm in diameter and cervix with more than 7.5 cm in diameter between 20 and 33 days postpartum (DPP) in cows with suffering from clinical endometritis were associated with a decrease in pregnancy rate. Kasimanickam *et al*. (2004) classified animals with UD and CD greater than three cm to be positive for endometritis at 33 to 47 DPP.

Materials and methods

The study was conducted at University Livestock Farm and Fodder Research Development Scheme, Mannuthy, Thrissur, Kerala from September 2018 and June 2019. The study included 20 crossbred dairy cows of three to 12 years of age in their first to seventh parity, which were clinically normal and without any postpartum complications. The animals were subjected to TRUS and EC.

A balanced feeding protocol was followed consisting of green and dry fodder along with concentrates and mineral mixture according to the Nutrient Requirements of Animals – Cattle and Buffalo (ICAR-NIANP), (2013). Pregnant animals were dried off at seventh month of gestation and maintained in a separate shed. The practice of weaning of calves immediately after parturition was followed and cows were milked twice daily.

Transrectal ultrasonography was performed on 30 and 40 DPP, with a real time colour Doppler ultrasound scanner (MyLab™ Gamma, Esoate SpA, Italy) equipped with liner array, 5-10 MHz frequency transrectal transducer to determine presence of FIU, evaluate the echogenicity of the contents if any and to measure the diameters of uterine horn and cervix.

Cytological studies of the uterus were carried out on 30 and 40 DPP using modified human Pap smear cytobrush, where the handle of brush was shortened to nearly 2cm, heat fixed to stylet of AI gun, withdrawn

to barrel and covered with plastic AI sheath with widened tip. The modified cytobrush was prepared aseptically under laminar air flow. The cows were restrained and vulva and perineum were cleansed to minimize contamination. The cytobrush was introduced into the vagina and by per rectal manoeuvring it traversed the cervix and reached the uterus. Once inside the uterus the stylet was pushed forwards to expose the cytobrush and rolled on to the uterine wall a full 360 degrees clockwise to obtain cellular material from the endometrium. Slides were prepared by rolling the cytobrush onto clean glass microscopic slides which were air-dried and stained using Field Stain.

The EC smear was evaluated using a microscope at 400 X magnification to identify individual cell types, including endometrial epithelial cells and polymorphonuclear (PMN) cells. The PMN cells count were expressed as the per cent of PMN cells counted out of the 100 cells (including PMN cells and endometrial cells). Cows were categorized into two groups *viz*., SCE - positive or negative on the basis of percentage of PMN cells. The animals with more than eighteen per cent PMN cells on days 21- 34 and more than 10 per cent on days 33- 47 were considered positive for subclinical endometritis (Kasimanickam *et al*., 2004).

The data obtained were subjected to statistical analysis using Repeated Measures ANOVA, Cochran's Q test and Receiver Operating Characteristic (ROC) Curve using SPSS 24.

Results and Discussion

The objective of the present study was to compare TRUS and EC by cytobrush technique for the diagnosis of subclinical endometritis in postpartum cows. Using cytobrush technique (EC) nine out of the 20 animals were diagnosed as SCE positive with more than 18 per cent PMN cells on 30th day of observation.

The Mean (± SE) percentage of PMN cells in normal and SCE cases are summarized in table 1. In normal animals the values were 1.82 ±0.58 and 1.00 ± 0.56 and in SCE group values were 20.00 ± 0.64 and 11.00 ± 0.62 on 30 and 40 DPP, respectively.

On statistical analysis there was significant difference in the PMN percentage (Fig. 1) between SCE positive and normal groups in both the days of observation and also there was a significant reduction in the PMN percentage from days 30 to 40 in SCE group (Table 1).

This was in par with the studies by Kasimanickam *et al*. (2004) who used ROC analysis to identify the PMN per cent above which fertility was significantly reduced and

Table1. Mean (± SE) of PMN cells on day 30 and 40 postpartum

Group	Per cent of PMN cells	
	Normal (n = 11)	SCE (n = 9)
Day 30	1.82±0.58^{Ab}	20±0.64^{Aa}
Day 40	1±0.56^{Ab}	11±0.62^{Ba}

(a,b Means bearing different superscripts within rows differ significantly at 5% level A,B Means bearing different superscripts within columns differ significantly at 5% level)

Table 2. Relationship between fluid in uterus and subclinical endometritis on 30 and 40 DPP

Group	Normal (n = 11)		SCE (n = 9)	
	Day 30	Day 40	Day 30	Day 40
FIU	5 (45.45)	6 (54.54)	6 (66.67)	6 (66.67)

Table 3. Mean (± SE) of Cervical diameter (mm) and Uterine horn diameter (mm) in normal and sub clinical endometritis positive animals

Group	Cervical diameter (mm)		Uterine horn diameter (mm)	
	Normal (n= 11)	SCE (n = 9)	Normal (n= 11)	SCE (n = 9)
Day 30	29.62±0.77	28.96±0.84	14.77±0.52	15.23±0.74
Day 40	28.68±0.85	29.56±0.67	14.83±0.55	15.35±0.69

Measured at 5% level of significance

arrived at 18 per cent for 20–33 days postpartum and 10 per cent for 34–47 days postpartum.

By performing TRUS, on day 30, six animals in SCE group and five animals in normal group had FIU and on day 40, six animals in SCE group and six animals in normal group had FIU. The percentage of animals with FIU was similar in both groups. This was in contrast to Dourey *et al*. (2011) who reported that there was a positive correlation between the quantity of uterine fluid and PMN percentage on four weeks postpartum, but it did not affect the interval from calving to first ovulation (Table 2).

The mean (± SE) of CD (mm) in the normal group was 29.62 ± 0.77 and 28 ± 0.85

Fig. 1 Microphotograph of endometrial cytology (PMN cells marked by arrow), 400X

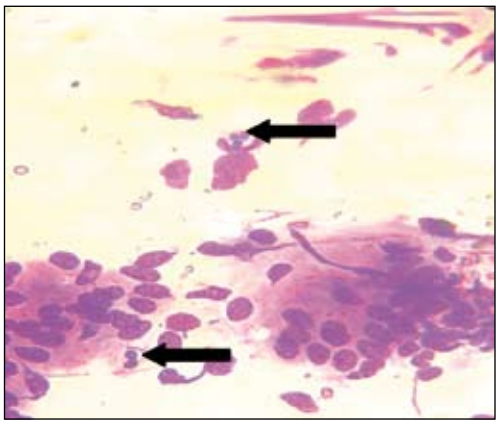


Fig. 2 Cervical Diameter (CD) at 30 and 40 DPP

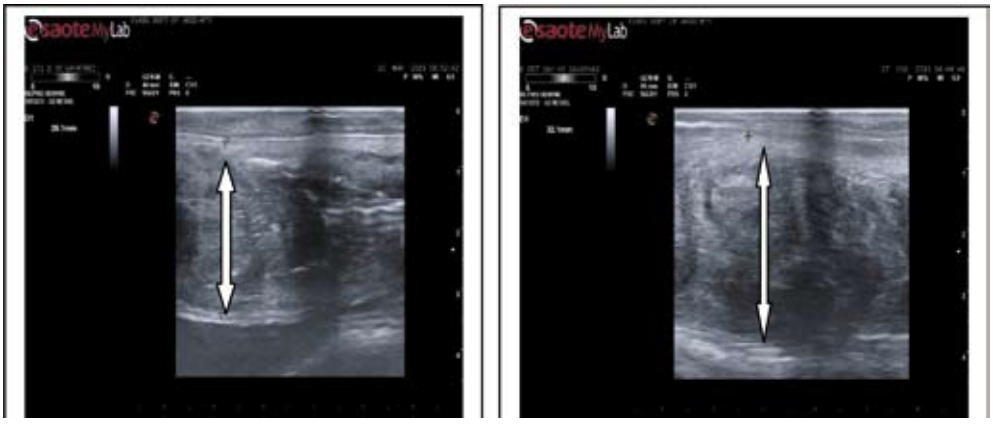
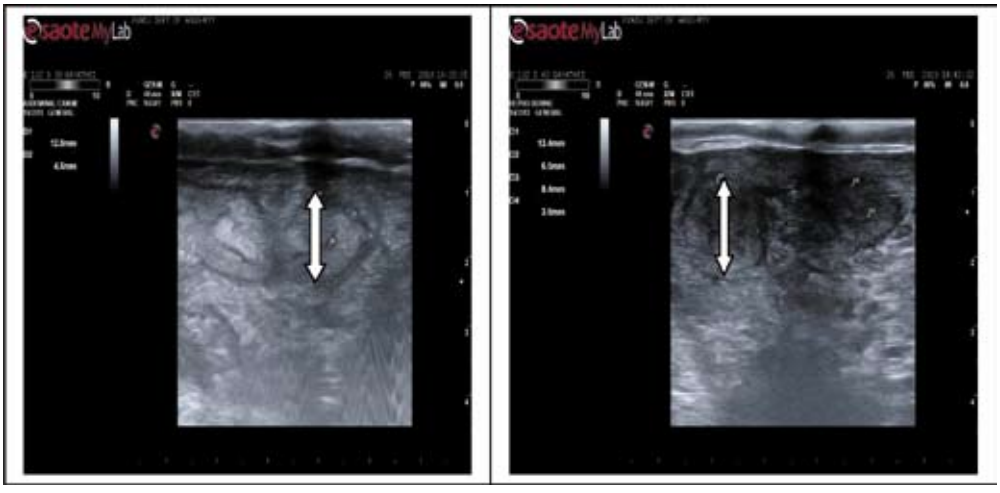


Fig. 3 Uterine horn diameter (UD) at 30 and 40 DPP



and in SCE group was 28 ± 0.96 and 29.56 ± 0.67 on 30 and 40 DPP, respectively (Table 3, Fig. 2).

On statistical analysis there was no significant difference between SCE and normal groups in both the days of observation. The cut off point for cytological endometritis positive was greater than three cm. All the animals had a CD less than three cm by day 30 irrespective of the group. Similar works were done in pure bred cows where the size of cervix in normal condition was considerably large (Kasimanickam *et al*., 2004).

The mean (± SE) of uterine horn diameter (UD in mm) in the normal group was 14.77 ± 0.52 and 14.83 ± 0.55 and in SCE group was 15.23 ± 0.74 and 15.35 ± 0.69 on 30 and 40 DPP, respectively (Table 3, Fig. 3). On statistical analysis there was no significant difference between SCE and normal groups on both the days of observation. Similarly, Mateus *et al*. (2002) also reported that changes in uterine diameter are identifiable only in severe endometritis and such changes are negligible in SCE.

Statistical analysis of the different parameters was performed using Cochran's Q test and Receiver Operating Characteristic (ROC) curve. On using Cochran's Q test for comparing the efficacy of FIU, CD and EC in the diagnosis of SCE, there was no significant difference on both 30 and 40 DPP.

However, on using Cochran's Q test for analysing the efficiency of FIU, UD and EC, the diagnosis based on UD was found to be significantly different from that of EC on both 30 and 40 DPP. Hence, its efficiency in diagnosing SCE is inferior in the present study.

On ROC curve analysis by keeping EC as gold standard, on day 30, FIU 30 has a sensitivity of 66.7 per cent and a specificity of 54.5 per cent. Area under curve (AUC) was 60.6 per cent which indicates that this model has less to moderate predictability.

CD has poor sensitivity (44.4%) and specificity (54.5%). AUC is small and near to random (49.5%) and has poor predictability.

UD has poor sensitivity (0%) and high specificity (100%). AUC is small and near to random (50%). Hence, this criterion is least valuable for the diagnosis of SCE.

On ROC curve analysis keeping EC as standard on day 40, FIU has a sensitivity of 71.4 per cent and a specificity of 46.2 per cent. AUC was 58.8 per cent which indicates that this model has low to moderate predictability.

CD has poor sensitivity (28.6%) but moderate specificity (69.2%). AUC is small and near to random (48.9%) and has poor predictability.

UD has poor sensitivity (0%) and high specificity (100%). AUC is small and near to random (50%). Hence, this criterion has low value for the diagnosis of SCE.

In the present study, endometrial cytology (EC) was kept as gold standard and TRUS parameters like UD, CD and FIU were compared. It was found that FIU could be used for the diagnosis of SCE with a sensitivity of 66.7 per cent and specificity of 54.5 per cent, when compared to EC, on day 30 and a sensitivity of 71.4 per cent and specificity of 46.2 per cent for day 40. However, FIU was influenced by the stage of oestrous cycle. The sensitivity and specificity obtained for FIU was greater than that reported by Drillich *et al*. (2004) (sensitivity of 57.7% and a specificity of 40.5%) where the presence of ultrasonographically detectable uterine fluid and endometrial cytology using cytobrush (EC) were compared using EC as the gold-standard in animals 21 -27 DPP.

This was also in accordance with Arias *et al*. (2018) who reported that the ultrasonographic detection of intrauterine fluid and evaluation of uterine diameter could be applied as a method to diagnose SCE and that a small amount of FIU or thickened uterine walls could be considered signs of endometrial inflammation.

In the present study there was no significant difference in cervical and uterine

horn diameter between the normal and SCE groups during both the days of study. Diagnosis using CD at 30 and 40 DPP had a sensitivity of 44.4 per cent and 28.6 per cent and a specificity of 54.5 per cent and 69.2 per cent respectively with EC as gold standard. Uterine horn diameter had zero sensitivity on both the days of observation.

The cut-off point for diagnosing an animal as SCE positive was taken according to the study by Kasimanickam *et al.* (2004) where a uterine horn diameter and cervical diameter greater than three cm was considered positive for diagnosing cytological endometritis in apparently normal animals. However, all the animals in the present study had a cervical diameter and uterine horn diameter less than 3 cm by day 30. This could be attributed to the fact that the present study was done in crossbred dairy cows which had a notably smaller uterine and cervical size when compared to the purebred cows used in the reference studies. Hence, the diagnostic parameters involving TRUS are of inferior value for the precise diagnosis of SCE in cross bred dairy cows when compared to EC in the present study.

Conclusion

The present study suggested that the TRUS parameters like uterine horn diameter, cervical diameter and fluid in uterus was found to be less sensitive and specific for the diagnosis of subclinical endometritis when compared to endometrial cytology. Further, in order to fix a cut off value for CD and UD in crossbred dairy cows detailed studies involving more number of animals may be required so as to achieve a statistically significant value in this regard. In SCE the changes in the uterus are minute and its detection by ultrasonography is subjective to error and requires special techniques for diagnosis such as endometrial cytology. In the present study, the cytological samples were collected using modified cytobrush and the PMN percentage was significantly different in animals with SCE. Such precise changes may not be detected in terms of uterine horn diameter and cervical diameter using TRUS. However, FIU had more sensitivity than cervical diameter and uterine horn width. Therefore, when ultrasonography is used as a supporting diagnostic aid along with cytology, it is of greater value in the accurate diagnosis of subclinical endometritis.

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RESEARCH ARTICLE
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Comparative analysis of closed and open-cervix canine pyometra*

V.K. Vidya[1], M.P. Unnikrishnan[2], M.O. Kurien[3], C. Jayakumar[4] and Surya Sankar[5]
Department of Animal Reproduction, Gynaecology and Obstetrics,
College of Veterinary and Animal Sciences, Mannuthy, Thrissur- 680 651
Kerala Veterinary and Animal Sciences University

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Abstract

Canine pyometra is the most common uterine disease in intact, sexually mature female dogs. The disease is broadly classified as open and closed type, based on the patency of cervix. The study was conducted to evaluate the changes in clinical, physiological and haemato-biochemical parameters in open and closed-cervix pyometra. Physiological parameters were not altered among pyometra-affected dogs. Vomiting, dehydration and dullness were more frequently noticed in open-cervix pyometra cases. Significantly higher total leucocyte count and band cell per cent and significantly lower total erythrocyte count was recorded among dogs with open-cervix pyometra than closed-cervix pyometra. The present finding is contradictory to most of the previous reports.

Keywords: *open-cervix pyometra, closed-cervix pyometra, dog, haemato-biochal parameters*

Canine pyometra is a reproductive disease characterised by bacterial infection and inflammation, with pus accumulating in the uterus, in combination with systemic illness (Hardy and Osborne, 1974). The disease is mainly encountered among middle-aged to older dogs, usually during diestrus and affects over 25 per cent of all intact female dogs before 10 years of age, with mortality rate of 3-4 per cent (Egenvall *et al.,* 2001). High serum progesterone concentration during diestrus will promote bacterial adherence and growth in the endometrial epithelium, finally ending up in the most severe end stage condition known as pyometra (Hagman and Kuhn, 2002).

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1. *MVSc Scholar and Corresponding author Email id: vidyaviswanath22@gmail.com 9633888658*
2. *Assistant Professor, Centre for Pig Production and Research, Mannuthy, Email id: unnikrishnan@kvasu.ac.in*
3. *Professor and Head (Retd.)*
4. *Assistant Professor and Head (i/c)*
5. *Assistant Professor, Department of Veterinary Microbiology*

Endotoxin, a lipopolysaccharide part of outer membrane of the cell wall of *Escherichia coli* and other Gram-negative bacteria, when released into circulation, as the bacteria grows or when destroyed, is thought to be responsible for the systemic symptoms of pyometra and sepsis in dogs (Asheim, 1965). The sepsis and endotoxeamia will alter the function of vital organs like liver, kidney and bone marrow and cause changes in haemato-biochemical parameters (De Schepper *et al.,* 1987). Wide variations in haematological and biochemical variables are reflections of systemic involvement of pyometra (Hagman, 2004).

Two forms of pyometra based on the cervical patency are recognised: open-cervix pyometra in dogs presented with vaginal discharge and closed-cervix pyometra in animals presented without vaginal discharge (Smith, 2006). More serious illness is reported among the dogs having closed- cervix pyometra, with pus and bacterial products retained in the uterus (Macphail, 2013). Physiological and haemato-biochemical changes in pyometra are considered significant to assess the severity of the disease condition (Singh *et al.,* 2006).

The aim of this study was to investigate the degree of severity among dogs with open and closed-cervix pyometra based on changes in physiological and haemato-biochemical parameters.

Materials and methods

Female dogs presented to University Veterinary Hospitals, Kokkalai and Mannuthy, attached to College of Veterinary and Animal Sciences, Mannuthy, Kerala were utilized for the study. Pyometra condition was diagnosed based on history, detailed clinico-gynaecological and ultrasonographical examination. Those having vaginal discharge on presentation were considered as open (Group I) and those without vaginal discharge were identified as closed-cervix (Group II) pyometra, while healthy dogs in diestrus were selected as control (Group III). Each group consisted of 14 female dogs each.

Signalment, anamnesis with special emphasis to detailed reproductive history, clinical signs and physiological parameters *viz.* rectal temperature (°F), respiratory rate (per minute) and heart rate (per minute) were recorded. Peripheral blood samples were collected and haematological parameters like total erythrocyte count (TEC, $\times 10^6/mm^3$), total leucocyte count (TLC, $\times 10^3/mm^3$), thrombocyte count (PLT, $\times 10^3/mm^3$), haemoglobin concentration (Hb, g/dL) and volume of packed red cells (VPRC, per cent) were recorded using automatic analyser (Mythic 18 Vet, Woodley, Switzerland). Blood smears were prepared after collecting blood by puncturing ear tip and stained by Leishman's stain for differential leucocyte count (DLC). Serum, after separation from whole blood, were centrifuged and a minimum of 1.5 mL serum sample was utilised for biochemical analysis of blood urea nitrogen (BUN, mg/dL) and creatinine (mg/dL) by using semi-automatic analyser (Master T biochemistry analyser, Hospitex diagnostics, Italy).

Analysis of variance was done to pyometra condition was diagnosed physiological and haematolo-biochemical compare parameters between groups.

Results and Discussion

Majority of pyometra affected dogs in group I and II were nulliparous dogs (64.29% each). Higher occurrence of pyometra observed in nulliparous dogs were reported earlier also (Unnikrishnan, 2018). In nulliparous dogs, repeated progesterone exposure during each dieostrus might lead to gradual development of CEH (Borrensen, 1979); subsequent fluid accumulation and bacterial infection would finally bring about pyometra (Hardy and Osborne, 1974).

Clinical findings in pyometra-affected dogs are presented in Table 1. Vaginal discharge, anorexia, dullness, dehydration, vomiting, polyuria and pale mucous membrane were the most frequently noticed clinical signs in Group I, whereas anorexia, dullness, congested mucous membrane, and polyuria were the predominant signs among Group II dogs. Clinical signs noticed in pyometra cases such as vomiting, anorexia and polyuria were

reflecting the systemic involvement of the disease. Gastro-intestinal disturbances are related to the adverse effects of endotoxemia and the presence of vomiting and diarrhoea indicates its severity (Hardie and Kruse- Elliott, 1990). Although Jitpean *et al.* (2017) reported more severe clinical signs among closed-cervix pyometra; in the present study, severe clinical signs were expressed more among dogs having open-cervix pyometra.

Data regarding physiological and haemato-biochemical evaluation of Group I, II and III dogs are presented in Table 2.

Temperature, heart rate and respiratory rate of dogs with either open or closed-cervix pyometra did not significantly differ from control. Similar observations were also made by Lakshmikanth *et al*. (2016). Hagman (2012) stated that higher body temperature, respiratory rate and heart rate were indicative of severe uterine inflammation, septicaemia, bacteraemia or a systemic inflammatory response in canines due to effects of endotoxins released from circulating bacteria. Findings in the present study indicate that endotoxemia was absent or not severe enough to cause changes in the physiological parameters.

Leucocytosis was noticed among pyometra-affected dogs; the value was highest among Group I and lowest in Group III; significant difference existed between groups. Similar changes were recorded for band cell per cent also, with significantly highest value among group I and lowest in Group III. Values among pyometra-affected dogs were higher than normal physiological limits. The findings are suggestive of inflammatory response among pyometra-affected dogs and higher degree of inflammatory response was noticed among dogs with open-cervix pyometra. The present observation differs from the findings of Jitpean *et al.* (2017), who reported that leucocytosis, neutrophilia and monocytosis were more commonly found in dogs with closed-cervix pyometra.

Neutrophil per cent was highest among Group II and lowest among Group III; neutrophilia was noticed among Group II and was significantly higher than Group III but did not differ from Group I. The findings are suggestive of immune response to infection, among pyometra-affected dogs. Leucocytosis with neutrophilia and left shift in pyometra might be due to aggressive bone marrow response on account of increased stress on immune mechanism and diffused suppurative inflammation of uterus to combat the infection (Kustritz, 2005).

The per cent of lymphocytes was lowest among Group II and highest among Group III; although values were within normal range among all the groups, per cent among pyometra-affected groups were in lower physiological limits and significantly differed from control animals. Lymphopenia among pyometra-affected dogs were reported earlier also by Singh *et al.* (2006) and lymphopenia was either due to suppression of immune system, caused by endotoxaemia or due to an absolute neutrophilia caused by severe suppurative inflammation of the uterus.

Table 1. Per cent of dogs exhibiting different clinical signs, among dogs affected with open and closed -cervix pyometra

Clinical signs	Group I (Open-cervix pyometra)	Group II (Closed-cervix pyometra)
Presence of vaginal discharge	85.71	0
Anorexia	78.57	100.00
Dullness	78.57	100.00
Dehydration	78.57	35.71
Vomiting	64.29	28.57
Polyuria	57.14	42.85
Pale mucous membrane	50.00	28.57
Congested mucous membrane	35.71	57.14
Polydipsia	14.28	0

Table 2. Physiological and haemato-biochemical parameters among pyometra-affected and control dogs

Parameters	Group I (Open-cervix pyometra)	Group II (Closed-cervix pyometra)	Control	F value	p-value
Temperature (°F)	102.40±0.42	101.94±0.33	101.51±0.22	1.83	0.17
Heart rate (per minute)	105.00±5.69	95.93±4.05	90.71±3.35	2.62	0.09
Respiratory rate (per minute)	25.07±0.70	24.48±1.04	23.71±0.66	0.72	0.49
TLC ($\times10^3$/mm^3)	39.01±5.43^a	24.98±3.90^b	10.7±0.62^c	13.35	<0.001
Band cell (%)	6.79±0.83^a	3.36±0.41^b	1.00±0.21^c	28.25	<0.001
Neutrophil (%)	74.81±2.41^{ab}	78.91±1.53^a	70.89±2.26^b	3.64	0.04
Lymphocyte (%)	17.44±2.01^b	14.45±1.10^b	23.58±1.95^a	7.20	<0.001
Monocyte (%)	7.75±0.61^a	6.64±0.60^{ab}	5.54±0.46^b	3.88	0.03
TEC ($\times10^6$/mm^3)	3.87±0.28^c	4.76±0.32^b	5.66±0.25^a	9.88	<0.001
Hb (g/dL)	9.01±0.60^b	10.52±0.82^b	12.42±0.45^a	7.29	<0.001
VPRC (%)	26.23±2.13^b	27.06±1.88^b	32.85±1.33^a	3.96	0.03
PLT($\times10^3$/mm^3)	173.21±22.30^b	213.04±34.20^{ab}	289.93±37.17^a	3.46	0.04
BUN (ng/dL)	21.16±4.81	22.05±6.86	15.28±0.59	0.58	0.57
Creatinine (ng/dL)	1.76±0.30	1.69±0.45	1.05±0.07	1.55	0.22

Monocyte count was within normal range among all the three groups, although it was significantly higher among Group I than controls. Similar finding was reported by Lakshmikanth *et al.* (2016). Monocytosis among pyometra-affected dogs was described an indication of chronicity of the suppurative process (Singh *et al*., 2006).

The TEC was highest among Group III and lowest among Group I; significant difference existed between groups. Among Group I and II, values were lower than normal physiological range, indicating the existence of anaemia among pyometra-affected dogs, the intensity of which was highest among dogs with open-cervix pyometra. Since endotoxin- mediated suppression of bone marrow and shortening of life of circulating erythrocytes are attributed as reasons for low TEC (Hagman *et al.,* 2009), it may be assumed that, in the present study, effect of endotoxemia was more pronounced in open-cervix pyometra group.

The Hb concentration was highest among Group III and lowest among Group I; the values among pyometra-affected dogs were lower than normal physiological range and significantly differed from control group. In pyometra affected dog, low level of Hb was due to iron deficiency as a consequence of iron sequestration in the bone marrow and withdrawal of iron from the normal erythropoiesis (Grimes and Fry, 2014).

The VPRC per cent was highest among Group III and lowest among Group I; the values among pyometra-affected dogs significantly differed from control group. The VPRC per cent among pyometra-affected dogs are suggestive of marginal anaemia. Low VPRC might be a reflection of reduced TEC, caused by toxic suppression of the bone marrow and consequent reduction in erythropoiesis (Unnikrishnan, 2018).

Although platelet count was within physiological limits among all the groups, value was highest among Group III and lowest among Group I, with significant difference between

these values. Values among pyometra-affected dogs were in the lower limits of physiological range, especially among open-cervix pyometra group. Thrombocytopenia among pyometra-affected dogs was explained earlier (Hagman, 2004). Marked thrombocytopenia in pyometric dogs might be due to endometrial bleeding or decreased production of thrombocytes in the bone marrow, mediated by endotoxin through production of thrombocyte activation factor. Lower platelet count might also occur as a result of disseminated intravascular coagulation (Sabine, 2015). Values in the lower physiological range among pyometra-affected dogs in the present study indicate that dogs were not under severe toxaemia.

Serum creatinine and BUN concentrations were within the normal range among all the groups and no significant difference existed between groups. The observation was similar to previous reports (Lakshmikanth *et al*., 2016; Jitpean *et al.*, 2017). Altered functions of organs such as kidney and liver were indicated by increased creatinine and BUN concentrations, hypoalbuminemia and proteinuria, among pyometra affected dogs (Maddens *et al*., 2011)

In the present study, no differentiation in physiological parameters could be made between open and closed-cervix-pyometra. Similarly, no differentiation could be made between open and closed-cervix pyometra, based on neutrophil, lymphocyte, monocyte counts and VPRC per cent. The serum creatinine, BUN and Hb concentration as well as platelet count also did not reveal any variation between open and closed-cervix pyometra. Volpato *et al*. (2012) reported that haematological and biochemical parameters had no significant difference between open and closed-cervix pyometra. Lakshmikanth *et al.,* (2016) reported that haematological parameters like haemoglobin, PCV, neutrophil, lymphocyte, eosinophil, and band cell did not vary significantly between open and closed type of pyometra; however, the values differed significantly when compared with healthy control dogs. In the present study also, most of the haematological parameters differed significantly from the control group.

Clinical signs were more severely expressed among dogs with open-cervix pyometra. Also, TLC, band cell per cent and TEC indicates that inflammatory response is more among dogs with open-cervix pyometra than closed cervix-pyometra. These findings are contradictory to most of the previous reports (Yu, 2012; Jitpean *et al.,* 2017). This could probably be due to many of the cases presented as open-cervix pyometra in the study might be closed cases initially, which might have been noticed by the owners only when the vaginal discharge commenced. Failure to recognise early signs of closed-cervix pyometra by the owner would lead to delayed presentation of cases; by this time, relaxation of cervix due to increased intrauterine pressure could occur and subsequently being classified as open- cervix cases, by the clinician (Anna *et al*., 2014).

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A study on the factors influencing resumption of postpartum ovarian activity in crossbred cows*

K. Sonam[1*], K. Promod[2], Leeba Chacko[3], K.C. Bipin [4], Lijo John[5]
Department of Animal Reproduction, Gynaecology and Obstetrics, College of Veterinary and Animal Sciences, Pookode, Wayanad, Kerala Veterinary and Animal Sciences Univesity.

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Abstract

The aim of the study was to identify the factors influencing postpartum resumption of ovarian activity in crossbred cows. Sixty crossbred cows on day 30 postpartum were selected and the details of age, parity, milk yield, peripartum and postpartum complications were recorded. Animals were examined per rectally from 30 to 90 days postpartum at 12 days interval for the presence of corpus luteum (CL) of any stage during the period of study. Serum progesterone concentrations were estimated and cows which resumed ovarian activity (progesterone concentration >1 ng/mL) at early and late postpartum periods were identified. Association of age, parity, BCS, milk yield and reproductive complications with postpartum resumption of ovarian activity (ROA) at varying periods was analysed. No significant association between ROA and variables such as age, parity. milk yield and reproductive complications were observed. BCS was significantly (P<0.01) associated with ROA. The higher number of animals with postpartum complications belonged to not resumed period than that of early and late resumed periods. The odds ratio for BCS indicated 6.46 times risk for ROA for every unit increase of BCS.

Key words: *Animal factors, postpartum dairy cows, resumption of ovarian activity*

The reproductive performance of dairy cows is dependent on the period at which the animal resumed its ovarian activity postpartum. Severe negative energy balance during postpartum period might increase the chance for the development of many metabolic disorders and reproductive complications. Major limiting factors which influence the postpartum ROA of dairy cattle are age, parity, BCS and milk yield. During the postpartum period timely uterine involution plays an important role in evoking ROA postpartum fertility. Uterine function is usually compromised in cattle

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1. *MVSc Scholar and Corresponding author Email id: sonamkathane1994@gmail.com*
2. *Associate Professor and Head*
3. *Assistant Professor*
4. *Assistant Professor, Department of Epidemiology and Preventive Medicine*
5. *Assistant Professor, Department of Veterinary Biochemistry*

by bacterial contamination of the uterine lumen after parturition, and pathogenic bacteria often persist, causing uterine disease, a key cause of infertility in cattle.

Uterine infections can delay the onset of postpartum ovarian cyclicity by delaying the initiation of folliculogenesis and suppressing the rate of follicular growth in dairy cows during the early puerperium by inhibiting LH release (Jainudeen and Hafez, 2000). Zraly *et al*. (1989) assumed that the delayed and asynchronous growth of follicles in cows with pathological puerperium was a consequence of disturbed repairing processes of endometrium and endocrine dysfunctions. Altered follicular responsiveness to gonadotrophic support through changes in metabolic hormones such as insulin-like growth factor I (IGF-I) and insulin may contribute to impaired function of dominant follicles at early postpartum. Mateus *et al*. (2002) reported that abnormal ovarian activity (prolonged anestrus, prolonged luteal phases, and ovarian cysts) in cows with severe endometritis.

Materials and Methods

The study was conducted at Instructional Livestock Farm Complex (ILFC), Pookode and Livestock Research Station (LRS), Thiruvazhamkunnu. Sixty crossbred cows aged between 3 to 10 years of age; 2 to 5 parity were selected for the study. Data regarding the milk yield, peripartum and postpartum complications of the selected animals were recorded. Observations on postpartum complications such as postpartum metritis, endometritis, cervicitis, vaginal prolapse and vaginitis were recorded.

The BCS of the cows was ascertained based on the fat deposition over certain areas of the body as per Smijisha (2012). Cows were subjected to clinico-gynaecological examination at 12 days interval from 30 to 90 days postpartum for the assessment of uterine involution, inflammatory changes and the presence of abnormal vaginal discharges to assess the reproductive health and ovarian functional status by detecting the presence of CL. Blood samples (5 mL) were collected by jugular venipuncture in clot activator blood collection vacutainers at 12 days intervals starting from day 30 till day 90 postpartum. Serum was separated by centrifugation at 1500 G for 10 minutes and stored in storage vials at –20°C until assayed for progesterone concentration. Progesterone estimation was carried out by using sandwich ELISA kit. The influence of age, parity, BCS, milk yield and reproductive complications for the ROA postpartum were analysed statistically. The ROA was assessed by the first rise in serum progesterone concentration above 1 ng/mL from days 30 to 90 postpartum. ROA was further divided into early resumption (before 42 days postpartum), late resumption (resumed between days 42 to 90 postpartum). The samples were subdivided into resumed or not resumed before 42 days postpartum to study the influence of different variables on early ROA by logistic regression analysis.

Result and discussion

Out of 60 animals selected 22 cows resumed cyclicity before day 42 postpartum and 38 animals not resumed cyclicity within 90 days postpartum (table 1).

Out of 22 animals which resumed cyclicity 8 (36.4%) cows were belonged to the age group < 4.5 years while 14 (63.6%) cows were ≥ 4.5 years of age. Out of 38 cows which did not resumed activity 15 (39.5%) were aged <4.5 years and 23 (60.5%) were aged ≥ 4.5 years. Nonsignificantly ($P > 0.05$) higher number of animals which did not resume cyclicity were aged ≥ 4.5 years (table 1)

Out of 22 animals resumed cyclicity 13 (59.1%) cows were belonged to the parity group ≤ 2 while 8 (40.9%) cows were > 2 parity. Out of 38 cows which did not resumed activity 18 (47.0%) were belonged to parity ≤ 2 and 20 (52.6%) were within the parity > 2. Nonsignificantly ($P > 0.05$) higher number of animals which did not resume cyclicity within the parity range > 2.

Out of 22 animals resumed cyclicity 10 (45.5%) cows were belonged to the BCS group ≤ 3 while 12 (54.5%) cows were > 3 BCS. Out of 38 cows which did not resumed activity 18 (47.0%) were belonged to BCS ≤ 3 and 20

(52.6%) were within the BCS > 3. Regression analysis of the data revealed a significant (P < 0.01) association of BCS with ROA postpartum was observed. It was found that higher the BCS range > 3, the number of animals which did not resumed cyclicity also increased. The odds ratio (6.46) for ROA was higher for BCS which indicated that there existed 6.46 times risk for every unit increase of BCS. However, the influence of age and parity were non significantly associated for development of postpartum ROA as its odds ratios were 0.704 and 0.290, respectively.

In the present study, age and parity of the selected animals were not significantly (P>0.05) influenced the postpartum ROA. Similar observations made in several earlier studies that postpartum ovarian activity was not influenced by age and parity and a prolonged quiescence of ovaries were observed in primiparous than in multiparous cows (Morrow *et al*., 1969; Bulman and Lamming, 1978). However, Opsomer *et al*. (2000) found parity as a clear risk factor for prolonged luteal phases in postpartum cows. They observed that cows that had calved 4 or more times were 2.5 times

Table 1. Significant factors affecting resumption of ovarian activity at different periods (< 42 days and > 42 days) postpartum (n=60)

Factors	Classification	Resumed before 42 days	Not resumed before 42 days	P value	Odds ratio	95% CI
Age	<4.5	8 (36.4%)	15(39.5%)	0.656^{ns}	0.704	0.151-3.288
	≥ 4.5	14 (63.6%)	23 (60.5%)			
Parity	≤2	13 (59.1%)	18 (47.0%)	0.107^{ns}	0.290	0.064-1.307
	>2	8 (40.9%)	20 (52.6%)			
BCS	≤ 3.0	10 (45.5%)	18 (47.4%)	0.003**	6.467	1.923-21.742
	>3.0	12 (54.5%)	20 (52.6%)			
Total no. of cows (n=60)		22 (36.67 %)	38 (63.33%)			

ns – Non significant, ** indicate significant (P<0.01), CI- confidence interval

Table 2. Influence of milk yield and reproductive complications for the early resumption (before 42 days) of ovarian activity postpartum (n=60).

Factors	Classification	n	P value	Odds ratio	95% CI
Milk yield	As per records	60 (100.0 %)	0.938^{ns}	1.185	0.938-1.498
Reproductive complications	No complications	37 (61.67%)	0.686^{ns}	0.767	0.212-2.775
	With complications	23 (38.33 %)			

ns- nonsignificant

Table 3. Number and per cent of cows resumed and not resumed the ovarian cyclicity at different periods postpartum

	Parameters	Postpartum resumption of ovarian cyclicity			
without reproductive Complications	No. and % of animals	**Not resumed >90 days**	**Early resumed (<42 days)**	**Late resumed (42-90 days)**	**Total no. and % of animals**
		16 (43.2%)	13 (35.1%)	8 (21.6%)	37 (61.67%)
With reproductive Complications	No. and % of animals	11 (47.8%)	9 (39.1%)	3 (13.0%)	23 (38.33%)
Total no. and % of animals		27 (45.0%)	22 (36.7%)	11 (18.3%)	60 (100%)

more at risk than primiparous cows for ROA. Zain *et al*. (1995) remarked that increased parity adversely affected both uterine involution and ROA. They found that cows with a parity ≥ 3 resumed cyclicity later (30.3± 3.6 days) than that in primiparous cows (25.5±2.9 days).

Gillund *et al*. (2001) also reported that cows with a BCS of more than 3.5 before calving might have 2.5 times higher risk of developing ketosis which could interfere with ROA postpartum. However, Butler and Smith (1989) indicated that cows which had a decrease in BCS of one unit or more during the first five weeks postpartum had a prolonged interval to the first observed oestrus when compared with their herd mates which had a meagre decline in body condition. Similarly, Opsomer *et al*. (1998) also observed that decrease in body condition was an important risk factor for delayed ovulation and cows losing more BCS during the first and second month after calving were significantly more at risk (odds ratio at 30 and 60 days after calving was 18.7, and 10.9, respectively) of a delay in ovarian function in the immediate postpartum period. Hence, prepartum determination of BCS could provide the status of body reserves of dairy cows which could be utilized during the period of NEB. However, the influence of milk yield and reproductive complications was nonsignificantly associated for development of postpartum ROA as its odds ratios were 1.185 and 0.767, respectively.

Out of 37 animals without reproductive complications 16 (43.2 per cent) cows not resumed cyclicity whereas 13 (35.1 per cent) and 8 (21.6 per cent) cows resumed cyclicity at early and later periods, respectively (table 3). Out of 23 animals with reproductive complications 11 (47.8 per cent) cows not resumed cyclicity whereas, 9 (39.1 per cent) and 3 (13.0 per cent) cows resumed ovarian activity at early and late postpartum periods, respectively. No significant differences (P>0.05) between cows with and without reproductive complications for the postpartum initiation of ovarian activities could be obtained. However, among cows with reproductive complications, nonsignificantly (P>0.05) higher proportion of cows (47.8 per cent) not resumed cyclicity as compared to rest of the population.

Studies showed that higher number of animals without reproductive complications (16 cows; 43.2 per cent) also not resumed before day 42 postpartum as compared to that of early and late periods of resumption. The higher animals with postpartum complications (11 cows; 47.8%) also not resumed ovarian activity than that of early and late resumed periods.

Puerperal diseases have long been implicated in retarding ovarian activity. (Opsomer *et al.,* 2000) observed puerperal disorders also played a significant role in the postpartum ROA. Cows with abnormal calving later affected by puerperal disorders were, 3-3.6 times more at risk of resuming ovarian activity. They also mentioned that cows that retained fetal membranes more than 12 h were not significantly at risk. The occurrence of clinical diseases such as clinical mastitis, severe lameness, or pneumonia during the first month after calving, was a significant risk factor for developing delayed ovarian activity. Cows with these diseases during the first month after calving were 5.4 times more at risk of having a delayed ovarian resumption after calving.

Conclusion

In the present study, no significant association between age, parity, milk yield and reproductive complications with resumption of ovarian activity could be elucidated. However, higher BCS at calving is a risk factor for delayed onset of cyclicity which might be due to rapid mobilization of body fat and subsequent metabolic disorders related to postpartum. More number of animals with reproductive complications failed to resume ovarian activity postpartum. Data from larger population is warranted for associating the variables to reach more conclusive findings.

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RESEARCH ARTICLE
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Physico-chemical and structural attributes of meat from young and spent buffaloes*

S. Kiran Kumar[1], V.N. Vasudevan[2], S.Prajwal[3], T.Sathu[2], A. Irshad[2], C.Sunanda[4], S.Silpa[1], and M.Pavan[3]
Department of Livestock Products Technology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur. Kerala Veterinary and Animal Sciences University

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Abstract

The current study was carried out to determine the physico-chemical and structural attributes of buffalo muscles from two anatomical locations obtained from animals belonging to two different age groups. Six each of young (2-4 years) and spent (8-10 years) buffaloes were procured from University Buffalo Farm, Mannuthy and were slaughtered scientifically at Meat Technology Unit, Mannuthy. Each carcass was electrically stimulated. The longissimus dorsi (LNG) and Biceps femoris (BIF) muscles were harvested, connective tissue and fat removed, aged for 72 hours at 4±1°C and analysed for the parameters. The ultimate pH values did not differ significantly between the muscles as well as the age groups. Hunter L and a* values were significantly different between the muscles. Hunter L* and b* values had significant difference between the age groups. Myofibrillar fragmentation index and Warner bratzler shear force of LNG and BIF were significantly different between age groups as well as between muscles. With respect to structural characteristics, no significant variation was noticed in sarcomere length between the muscles and the age groups. Between two age groups, muscle fibre diameter of samples from spent animal group showed significantly higher values. Thus, the results indicate important differences in the quality attributes of buffalo meat of two different age groups, which may be reflected in their palatability and processing properties.*

Key words: *Physico chemical attributes, meat, spent buffaloes*

Buffalo meat has gained importance in the recent years because of expanding domestic market and export potential. Buffalo meat is comparable to beef in many of its physicochemical,

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1. *M.V.Sc. scholars (Corresponding author email : kiran2551994@gmail.com)*
2. *Assistant Professors*
3. *Ph.D. Scholar*
4. *Assistant Professor, Department of Statistics, CVAS, Pookode.*

nutritional and functional properties and sensory attributes. Furthermore, its use in meat processing is increasing because of its higher content of lean meat and lower fat. Most of the buffalo meat production in India is from animals which are spent and unproductive. However, buffalo male calf rearing for meat production has been mooted as a sustainable way of salvaging these otherwise neglected source of meat animals. Hence, it is pertinent to analyse and evaluate the physico-chemical and structural attributes of the meat obtained from young and spent buffaloes. The current study was thus carried out to compare the physico-chemical and structural characteristics of meat from young and spent buffaloes.

Materials and methods

Twelve buffaloes from the University Buffalo farm, Kerala Veterinary Animal Sciences University, Mannuthy were utilized in this study. The animals belonged to two age groups *viz.* 2-4 years (n=6) and 8-10 years (n=6). They were reared intensively under similar management practices with occasional periods of grazing. The animals were slaughtered at the multi-species abattoir of the Meat Technology Unit, Kerala Veterinary and Animal Sciences University, Mannuthy after 24 hours of fasting as per scientific slaughter procedures. Ante-mortem and post-mortem inspections were conducted for each animal. The carcasses were electrically stimulated (100-110V, 1.5 to 2 min) and *Longissimus dorsi* (LNG) and *Biceps femoris* (BIF) muscles were immediately harvested from each carcass by hot deboning. After removal of separable fat and the connective tissue, each muscle was packed in High Density Poly Ethylene (HDPE) pouches and aged for 72 h at 4±1°C (Samsung Digital Inverter Technology, India). After ageing, each muscle was portioned for analysis of physico-chemical and structural attributes. The muscles were further packed in LDPE pouches and stored at -18±2ºC until subsequent analysis which took place within one week of freezer storage. Meat was thawed at 4±1ºC for 12 h before assessment of the following parameters: Ultimate pH (O'Halloran *et al.*, 1997), colour (Hunter L*, a*, b* values), myofibrillar fragmentation index (Davis *et al.,* 1980), Warner-Bratzler shear force (Wheeler *et al.,* 1997), sarcomere length (Hostetler *et al.,* 1972) and muscle fibre diameter (Jeremiah and Martin, 1977).

Results and Discussion

Ultimate pH

The mean ultimate pH of LNG and BIF muscles from both the age groups is shown in Table 1. Among the two muscle samples from both the age groups, LNG from spent buffaloes had the highest ultimate pH (5.96±0.27). LNG and BIF muscles had no significant difference with respect to ultimate pH values between two age groups. There was no significant difference in ultimate pH between LNG and BIF within the same age group which is in agreement with the findings of Kandeepan *et al.* (2009). Higher pH of buffalo meat from both the age groups obtained in the current study when compared to some of the previous reports could be due to the slower decline of pH as reported by Neath *et al.* (2007).

Warner Bratzler Shear Force (WBSF)

Mean WBSF values (Newton) of LNG and BIF muscles from both age groups are shown in Table 1. Mean WBSF values of LNG and BIF muscles from young and spent buffaloes were 40.13±1.08N, 66.81±4.86, 56.90±1.10N and 89.34±5.89N, respectively. There was a significant ($p<0.01$) difference in WBSF values between the muscles in both the age groups, with BIF exhibiting significantly higher values. Similar results were found by Prajwal *et al.* (2007) who reported the highest

Table 1. Ultimate pH, Warner-Bratzler shear force (WBSF) and myofibril fragmentation index (MFI) of young and spent buffalo meat (Mean±S.E.)

Attributes	Muscles	Animal group		F-value (p-value)		
		Young	Spent	Between age groups	Between muscles	Age* muscle
pH	LNG	5.79±0.05	5.96±0.27	0.27^{ns} (0.61)	0.01^{ns} (0.94)	0.34^{ns} (0.57)
	BIF	5.86±0.051	5.868±0.13			
WBSF (N)	LNG	$40.13±1.08^{ax}$	$56.90±1.10^{bx}$	32.03** (0.00)	124.13** (0.00)	2.65^{ns} (0.13)
	BIF	$66.81±4.86^{ay}$	$89.34±5.89^{by}$			
MFI	LNG	$662.47±10.24^{ax}$	$723.17±6.92^{bx}$	39.72** (0.00)	12.701** (0.01)	0.12^{ns} (0.73)
	BIF	$772.98±12.57^{ay}$	$822.8±26.93^{by}$			

Means having different small letters as superscripts differ significantly: ab- across column, xy- across rows. *Significantly different at 5% level, ns- non-significant. LNG-*Longissimus dorsi,* BIF-*Biceps femoris*

Table 2. Hunter L* a* and b* values of young and spent buffalo meat (Mean±S.E.)

Attributes	Muscles	Animal group		F-value (p-value)		
		Young	Spent	Between age groups	Between muscles	Age* muscle
*L**	LNG	$32.62±1.2^{ax}$	$28.83±2.06^{bx}$	6.807* (0.02)	5.136* (0.047)	1.11^{ns} (0.316)
	BIF	$31.37±1.78^{ay}$	$25.39±0.66^{by}$			
*a**	LNG	$14.814±0.617^{x}$	$14.079±0.898^{x}$	0.329^{ns} (0.579)	13.99* (0.04)	0.224^{ns} (0.646)
	BIF	$11.94±0.745^{y}$	$11.849±0.467^{y}$			
*b**	LNG	$13.63±1.00^{a}$	$12.10±0.8^{b}$	5.622* (0.039)	1.532^{ns} (0.244)	0.191^{ns} (0.671)
	BIF	$13.08±0.51^{a}$	$10.94±0.49^{b}$			

Means having different small letters as superscripts differ significantly: ab- across column, xy- across rows. *Significantly different at 5% level, ns- non-significant. LNG-*Longissimus dorsi,* BIF-*Biceps femoris*

Table 3. Sarcomere length (SL) and muscle fibre diameter (MFD) (µm) of young and spent buffalo meat (Mean±S.E.)

Attributes	Muscles	Animal age		F-value (p-value)		
		Young	Spent	Between age groups	Between muscles	Age* muscle
SL	LNG	1.74±0.35	1.67±0.02	1.462^{ns} (0.254)	0.004^{ns} (0.954)	0.126^{ns} (0.730)
	BIF	1.76±0.06	1.65±0.11			
MFD	LNG	$54.57±0.86^{a}$	$70.26±2.18^{b}$	17.608** (0.002)	1.299^{ns} (0.281)	7.349^{ns} (0.022)
	BIF	$58.50±3.26^{a}$	$60.63±2.32^{b}$			

WBSF value for BIF among ten buffalo muscles studied. Significantly higher WBSF for BIF than LNG could be due to a more stable connective tissue. Between the two age groups also, WBSF of both the muscles differed significantly ($p<0.01$), with samples from the spent age group showing significantly higher values. Significant increase in WBSF in LNG of older buffaloes has been previously reported by Rao *et al.* (2009), which is due to the fact that collagen with in the muscle tissue develops more stable cross links as it matures with its obvious adverse effect on subjective and objective tenderness values (Weston *et al.* 2002).

Myofibrillar Fragmentation Index (MFI)

Mean MFI values of LNG and BIF muscles from both age groups are shown

in Table 1. Mean MFI values of LNG and BIF muscles from young and spent buffaloes were 662.47±10.24, 772.98±12.57, 723.17±6.92N and 822.8±26.93 respectively. There was a significant ($p<0.01$) difference in MFI values between the muscles in both the age groups, with BIF exhibiting significantly higher values. Similar observations were reported by Prajwal *et al.* (2017). Between the two age groups also, MFI of both the muscles differed significantly ($p<0.01$), with samples from spent buffaloes showing significantly higher values. Ilavarasan *et al.* (2016) reported similar significantly different MFI values for LNG from Toda buffaloes of young and adult age groups. Kandeepan *et al.* (2009) also reported lower MFI values (expressed as percentage) for meat from old (>10 years) than young (18 months) buffaloes.

Colour (Hunter L*, a* and b* values)

Hunter L* values

The Hunter L* values of LNG and BIF muscles from both age groups are shown in Table 2. Between the age groups, LNG (25.39±0.66) and BIF (28.83±2.06) from spent buffaloes had significantly ($p<0.05$) lower Hunter L* values compared to LNG (32.62±1.15) and BIF (31.37±1.78) from young buffaloes. Between the muscles, BIF from both the age groups had significantly ($p<0.05$) higher Hunter L* values than the LNG from both age groups. Being a locomotor muscle, BIF might have endured higher physical activity in the live buffaloes as compared to less physical activity of LNG resulting in lower myoglobin content (Dikeman and Devine, 2014).

Hunter a* values

The Hunter a* values of LNG and BIF muscles from both age groups are shown in Table 2. Hunter a* values for LNG and BIF from young and spent buffaloes were 14.81±0.62, 11.94±0.76, 14.08±0.90 and 11.85±0.47, respectively. Between muscles, a* value was significantly ($p<0.05$) higher for LNG than BIF in both the age groups. This is in agreement with the observation of Prajwal (2016). Between age groups, a* values of both LNG and BIF did not differ significantly because young buffaloes utilized in the current study were adult animals of 2-4 years with possible higher myoglobin content, which could have resulted in lack of any significant difference as compared to a* values of meat from spent animals.

Hunter b* values

The Hunter b* values of LNG and BIF muscles from both age groups are shown in Table 2. Hunter b* values for LNG and BIF from spent and young buffaloes were 12.10±0.80, 10.94±0.50, 13.63±1.01 and 13.08±0.51, respectively. Between muscles, Hunter b* values did not differ significantly in both the age groups. This observation differed from that of Prajwal (2016) who reported significantly different b* values between buffalo LNG and BIF from 4-6 years old buffaloes. This different observation in the current study when compared to above mentioned study could be due to different age groups of animals selected for evaluation. However, between age groups, both LNG and BIF from young buffaloes had significantly ($p<0.05$) lower b* values. This finding is in accordance with Rao *et al.* (2009) who also reported lower values for chroma (intensity of colour) for buffalo LNG muscles in 2-4 years old non-descript buffaloes when compared to samples that were obtained from animals aged between 6 months and 2 years.

Structural Characteristics

Sarcomere length

The mean sarcomere length (µm) of LNG and BIF muscles from both age groups are shown in Table 3. Mean sarcomere lengths of LNG and BIF muscles from young and

spent buffaloes were 1.74±0.35, 1.76±0.06, 1.67±0.02 and 1.65±0.11, respectively. There was no significant difference in sarcomere length between the muscles in both the age groups. Between two age groups also, sarcomere length values of both the muscles did not differ significantly with samples from younger age group showing slightly higher values. Ffoulkes(1992) reported a decrease in sarcomere length of buffalo muscle fibres with advancing age.

Muscle fibre diameter

Mean muscle fibre diameter (µm) of LNG and BIF muscles from both age groups are shown in Table 3. Mean muscle fibre diameter of LNG and BIF muscles from young and spent buffaloes were 54.57±0.86, 58.50±3.26, 70.26±2.18 and 60.63±2.32, respectively. There was no significant difference in muscle fibre diameter values between the muscles in both the age groups. Prajwal (2016) also observed no significant difference in muscle fibre diameter between buffalo LNG and BIF muscles. But between the two age groups, muscle fibre diameter of both the muscles differed significantly ($p<0.01$), with samples from spent buffaloes showing higher values. Similar observations were recorded by Kandeepan *et al.* (2009) and Ragab *et al.* (1966).

Means having different small letters as superscripts differ significantly: ab- across column, xy- across rows. *Significantly different at 5% level, ns- non-significant. LNG-*Longissimus dorsi,* BIF-*Biceps femoris*

Conclusions

The study was carried out to evaluate and compare physico-chemical and structural attributes of meat from young and spent buffaloes. The ultimate pH and Hunter L* and b* values had significant difference between the age groups. Myofibrillar fragmentation index and WBSF of LNG and BIF had significant difference between age groups as well as between muscles. With respect to structural characteristics, muscle fibre diameter of samples from spent animal group showed significantly higher values. Thus, the results indicate important differences in the quality attribute of buffalo meat of two different age groups, which may be reflected in their palatability and processing properties.

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RESEARCH ARTICLE
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Anatomical observations on the hair distribution patterns in the deer, goat and sheep

S. Maya[1], N. Ashok[2], K. M. Lucy[3], V. R. Indu[4], A. R. Sreeranjini[5], N. S. Sunilkumar[6] and K.B. Sumena[7]

Department of Veterinary Anatomy and Histology, College of Veterinary and Animal Sciences, Mannuthy. Kerala Veterinary and Animal Sciences University, Pookode.

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Abstract

Hair distribution patterns in the deer, goat and sheep were studied using skin samples collected from spotted deer brought for post mortem at College of Veterinary and Animal Sciences, from the Thrissur zoo and forest department and from goats and sheep freshly slaughtered at the Meat Technology Unit, Mannuthy. Samples of 1cm^3 were collected from 27 regions of skin, viz. muzzle, infraorbital, horn glands, dorsal face, lateral face, ventral face, ear pinna, dorsal neck, lateral neck, ventral neck, dorsal abdomen, lateral abdomen, ventral abdomen, dorsal forelimb, palmar, dorsal hindlimb, plantar, interdigital part of fore limb, interdigital part of hind limb, forelimb foot pad, hindlimb foot pad, inguinal, preputial scrotal regions of male dorsal thorax, perineum and dorsal nasal regions. The hair arrangement in the three species was simple, but arranged in groups. Mostly two to three hairs formed a group and they emerged out very closely but not from a single orifice unlike in the case of compound hairs. The muzzle region lacked hair on the rostral aspect and sparse wool hairs were found on the dorsal surface. Dorsal nasal and carpal regions consisted of dense population of short, stout hairs. Maximum hair density per microscope field under low power was noticed in the lateral aspect of neck, dorsal abdomen, palmar, interdigital aspect of hind limb, lateral abdomen and dorsal aspect of hind limb in deer. Hair was absent in the muzzle followed by dorsal face region in all three species in the present study. Maximum hair density per microscope field under low power was noticed on the dorsal aspect of fore limb, palmar aspect, pinna of ear, dorsal abdomen and interdigital space of hind limb in goat with minimum hair distribution on the ventral abdomen followed by lateral face region. Maximum hair density per microscope field under low power was noticed on the dorsal aspect of neck, interdigital space of fore limb, lateral aspect of neck and infraorbital in sheep with minimum hair distribution on the ventral abdomen, dorsal face and lateral face. In general, density of hair distribution was more in the deer than the goat and sheep.

Keywords: Deer, goat, hair distribution, sheep

1. *Professor and Head, E-mail: maya@kvasu.ac.in; 9446625160*
2. *Registrar and University Head , KVASU*
3. *Controller of Examinations, KVASU*
4. *Associate Professor*
5. *Associate Professor*
6. *Assistant Professor*
7. *Assistant Professor*

Animal species identification can be done based on many features including morphology of animal remains, particularly hair and bone. Hair morphology is an important tool that can be used to identify animal species (Yasser *et al.,* 2018).

Hair consists of two parts, root which is embedded in the dermis of the skin, and shaft which extends above the epidermis as a cylindrical structure. The hair shaft consists of three distinct morphological layers, medulla (the central layer), cuticle (the outer layer) and the cortex (between the medulla and the cuticle) (Debelica and Thies, 2009). Regional skin variations relating to the amount and type of hair coat, distribution and type of glands and skin thickness occur as functional adaptations to suit the organism to its environment (Banks, 1981). The objective of the present study was to investigate the differences in hair distribution pattern of skin in the three species of wild and domestic small ruminants.

Materials and Methods

Hair distribution patterns in deer, goat and sheep were explored using skin samples collected from six each of spotted deer brought for post mortem at the College of Veterinary and Animal Sciences, Mannuthy, from Thrissur zoo and forest department and of goat and sheep freshly slaughtered at the Meat Technology Unit, Mannuthy. Samples of 1cm^3 were collected from 27 regions of skin, *viz.* muzzle, infraorbital, horn glands, dorsal face, lateral face, ventral face, pinna ear, dorsal neck, lateral neck, ventral neck, dorsal abdomen, lateral abdomen, ventral abdomen, dorsal forelimb, palmar, dorsal hindlimb, plantar, interdigital part of fore limb, interdigital part of hind limb, foot pad of forelimb, foot pad of hind limb, inguinal , preputial , scrotal regions of male), dorsal thorax, perineum and dorsal nasal region.

Morphology of skin was studied by using a digital camera (Canon) with 5x zoom and 16 mega pixels. The gross morphological observations were studied under a stereo zoom microscope. Specimens for histological study were fixed in 10 per cent neutral buffered formalin (10% NBF), for 48 hours. The fixed specimens were processed for paraffin embedding (Luna, 1968). Further, serial sections of 5µm thickness were made and stained with Haematoxylin and Eosin for routine studies (Luna, 1968). Samples of 1 mm^3 size were fixed in 2.5% gluteraldehyde in 0.1 M phosphate buffer (pH 7.2) for 24 hrs at 4°C and processed for Scanning Electron Microscopy (SEM - Model: JOEL-JSM 5600) as per the standard procedures (Bozzola and Russell, 1998) at Ruska labs, College of Veterinary Science, Hyderabad.

Results and Discussion

Morphology: The hair arrangement in the three species in the present study was simple, but hairs were arranged in groups (Figs.1 to 4). Mostly two to three hairs formed a group and they emerged out very closely but not from a single orifice unlike in the case of compound hairs. This was in accordance with the observations of Eurell and Frappier (2013) and they stated that simple hair follicles were evenly distributed in ruminants and equines and were seen in groups of three in porcine. Variations in the thickness of the skin were also reported in different regions of the body, with the epidermis being thin in regions with heavy protective hair coat and thicker in non-hairy parts of the skin and at the muco-cutaneous junctions.

Histology: Maximum hair density per microscope field under low power was noticed on the lateral aspect of neck, dorsal abdomen, palmar, interdigital part of hind limb, lateral aspect of abdomen and dorsal aspect of hind limb with an average of 44, 25, 20, 16, 12 and 11 in deer.

Maximum hair density per microscope field under low power was noticed on the dorsal aspect of fore limb, palmar, pinna of ear, dorsal aspect of abdomen and interdigital space of hind limb with an average of 27, 19, 16, and 11 each in goat. Hair distribution was minimum on the ventral abdomen with two hair follicles per field in goat. This was followed by lateral aspect of face region with an average number of three in goat.

Maximum hair density per microscope field under low power was noticed on the dorsal

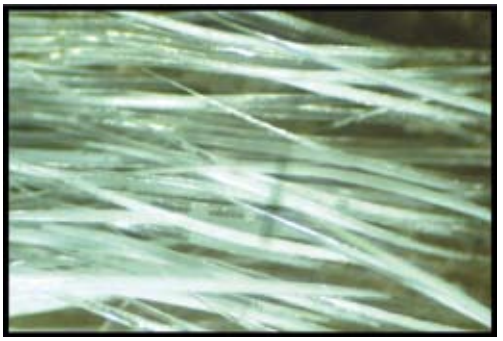

Fig. 1. Hair distribution on the pinna. Dorsal Aspect. 5 months–old male Crossbred Malabary Goat. Stereo zoom microscopy x 200

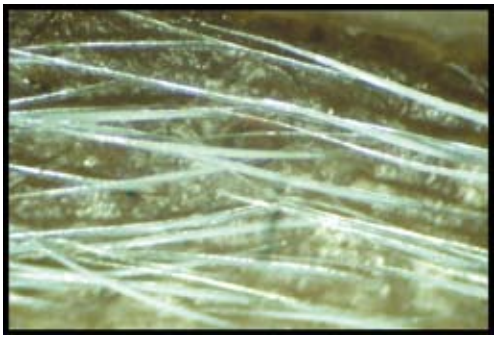

Fig. 2. Hair distribution on the pinna. Ventral Aspect. 5 months–old male Crossbred Malabary Goat. Stereo zoom microscopy x200

Fig. 3. Hair distribution on the abdomen. Dorsal Aspect. 6 year–old female Sambar deer. Stereo zoom microscopy x100

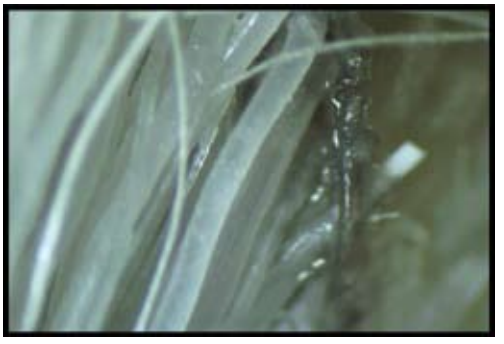

Fig. 4. Hair distribution on the abdomen. Dorsal Aspect. Adult male sheep. Stereo zoom microscopy x200

aspect of neck, interdigital aspect of fore limb, lateral aspect of neck and infraorbital with an average of 11 each in former two and 10 each in latter two each in sheep. Hair distribution was minimum on the ventral abdomen, dorsal and lateral aspect of face with three hair follicles per field in sheep.

In general, density of hair distribution was more in the deer (11.880) than the goat (9.714) and sheep (5.940) probably owing to an adaptation to the wild environment.

Among the three species studied, the muzzle region had the thickest epidermis. Adult deer had the greatest thickness for the region (619 µm) followed by goat (413 µm). Epidermis was thinnest in sheep (234 µm).

***Scanning Electron Microscopy*:** Scanning electron microscopic studies revealed that three hair types *viz.* primary, secondary and tertiary were present in all three species with higher hair density and less sloughing off in the deer (Fig. 5 to 8). Scale like cells covered the hair surface and there was a difference in width between hair types with a maximum in primary, decreased in secondary and tertiary (Fig. 6). The distance between every two successive scale margins were close in all the three species studied. The scales showed flattened edges in goat and sheep, but slight serrations were present in the deer.

Debelica and Thies (2009) also described the cuticle, the outermost layer of hair, as consisting of overlapping keratin scales. Two main patterns of cuticle scales were identified, the first being the imbricate, this includes ovate, acuminate, elongate, flattened

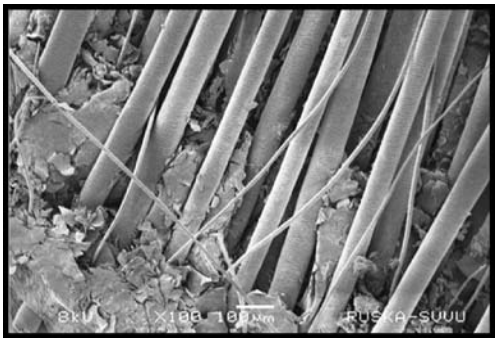

Fig. 5. Surface view of skin showing base of hair groups. Dorsal abdomen. Five- months old male sheep. SEM x 100

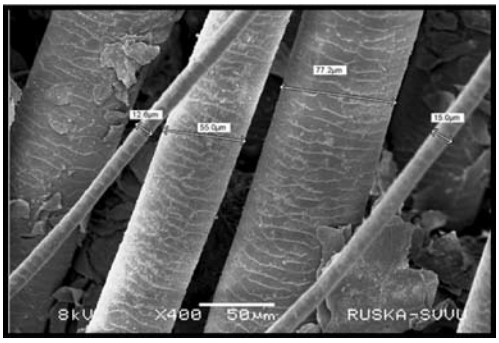

Fig. 6. Surface view of hair on Dorsal abdomen of Five- months old male sheep. SEM x 400

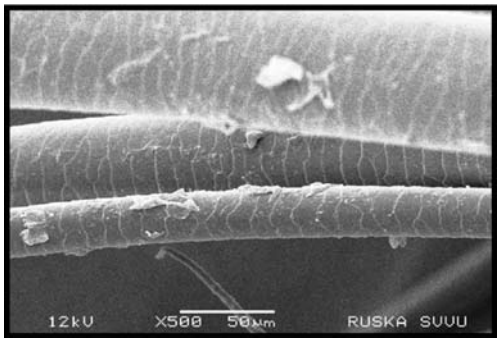

Fig. 7. Surface view of Dorsal abdomen of One week-old female deer. SEM x 500

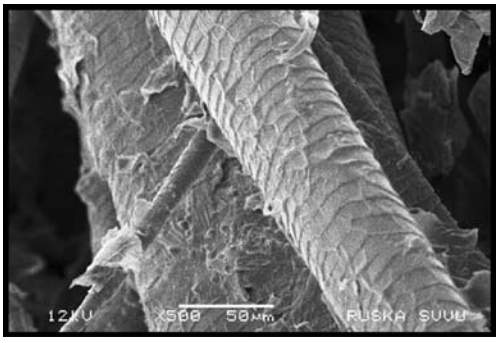

Fig. 8. Hair types. Surface view of Dorsal abdomen of Adult male sheep. SEM x 500

and crenate cuticles and the second being the coronal, which include simple, serrate or dentate cuticles. The distance between every two successive scale margins can be close, intermediate or wide, depending on the animal species.

According to Brunner and Coman (1974), the pattern of the cuticle scales, the type and the diameter of the medulla and/or the characteristics of pigmentation can be used for animal species identification as well as for differentiation between animal and human hair in forensic cases.

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Effect of coenzyme q10 supplementation on total antioxidant status and lipid peroxides levels in dogs with chronic valvular heart disease*

K. Revathi[1], N. Madhavan Unny[2], Usha Narayana Pillai[3], R. Uma[4] and S. Ajithkumar[5]
Department of Veterinary Clinical Medicine, Ethics and Jurisprudence,
College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, 680651
Kerala Veterinary and Animal Sciences University

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Abstract

Oxidative stress management has been found to have beneficial effects in the management of several disease conditions. A study was conducted to evaluate the efficacy of Coenzyme Q10 supplementation on the total antioxidant status and lipid peroxides levels in dogs with chronic valvular heart disease. Total antioxidant status was measured by Ferric Reducing Antioxidant Power assay and level of lipid peroxides in serum was determined by estimating malonaldialdehyde level. Ten dogs with chronic chronic valvular heart disease were administered with coenzyme Q10 @ 45mg q12h orally for 60 days along with the treatment for management of valvular heart disease. A significant decline in Ferric Reducing Antioxidant Power value was recorded at the end of the study. The malonaldialdehyde levels declined in animals with chronic valvular heart disease by day 60, though reduction was not statistically significant. The administration of Coenzyme Q10 in chronic valvular heart disease may be beneficial in improving the quality of life of the patient.

Keywords: *Dog, Coenzyme Q10, Chronic valvular heart disease, Oxidative stress*

Chronic valvular disease is the most common acquired heart disease in dogs. This disorder is considered to be a frequent cause of cardiac mortality in dogs (Haggstrom *et al*., 2004). Valve leaflets will undergo consequent before degeneration, deformation and thickening along with other senile changes of the body. Valvular affections reduce normal cardiac functions which in turn results in cardiac failure. Age related oxidative stress directly affects the cardiovascular system and it is assessed by measuring different biomarkers of oxidative stress. Coenzyme Q10, an antioxidant, is a cellular membrane stabilizer and free radical scavenger that suppresses the formation of reactive oxygen species during lipid peroxidation. Investigations on the role of antioxidant molecules in

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1. *MVSc Scholar*
2. *Associate Professor*
3. *Professor and Head*
4. *Assistant Professor, Department of Veterinary Biochemistry*
5. *Professor and Head, University Veterinary Hospital, Mannuthy*

the treatment of heart diseases in dogs could provide new therapeutic options for veterinary clinicians.

Materials and methods

Dogs brought to the University Veterinary Hospital, Kokkalai and Teaching Veterinary Clinical Complex, Mannuthy with cardiac ailments were screened for the study. Based on a detailed clinical examination along with electrocardiographic, radiographic and echocardiographic studies, chronic valvular heart disease was confirmed in 10 dogs. The animals were treated with furosemide @ 2mg/kg bodyweight q12h, enalapril @ 0.5mg/kg bodyweight q12h, pimobendan @ 0.25mg/kg body weight q12h orally and coenzyme Q10 @ 45mg q12h orally. Six apparently healthy adult dogs were taken as the control group. Oxidative stress was evaluated with the assessment of total antioxidant status and lipid peroxidation level on 0^{th}, 30^{th} and 60^{th} day of treatment.

Total antioxidant status (TAS) was measured by Ferric Reducing Antioxidant Power (FRAP) assay (Benzie and Strain, 1996). Level of lipid peroxides in serum was determined by the method of Yagi (1984) by estimating the malonaldialdehyde (MDA) level. Statistical analysis was done by one way ANOVA and paired't' test using SPSS version 24.0.

Results and discussion

Total antioxidant status of diseased animals was significantly high ($p \leq 0.01$) when compared to healthy animals on 0^{th} and 30^{th} day of treatment. Hetyey *et al.* (2007) made similar observations and reported a significantly higher FRAP value in dogs with dilated cardiomyopathy and mitral endocardiosis when compared to healthy animals. Increased FRAP value may be due to a higher level of uric acid as it contributes to 60 per cent of the fresh human blood plasma (Benzie and Strain, 1996). High uric acid concentration was observed in heart failure patients (Khan *et al*., 2017). In heart failure, increased production, reduced excretion or both will result in elevated uric acid levels. Increased abundance and activity of xanthine oxidase (XO) which is a contributor of reactive oxygen species in the failing heart coupled with elevated XO substrates and an increased conversion of xanthine dehydrogenase to XO are possible contributors of an elevated uric acid production (Hare *et al.,* 2003; Doehner and Anker, 2005).

From 0^{th} to 60^{th} day of treatment with coenzyme Q10, a significant decline ($p \leq 0.01$) in FRAP value from 1599.43±132.28 to 910.17±118.17µM /L was recorded. Maheshwari *et al.* (2014) and Sharma *et al.* (2016) reported on the ability of coenzyme Q10 to reduce uric acid levels. So the reduction in FRAP value after treatment with coenzyme Q10 may be attributed to the decrease in uric acid level or to reduced oxidative stress. Therefore, it can be concluded that the supplementation of coenzyme Q10 could reduce the oxidative stress associated with chronic valvular heart disease in dogs.

Serum MDA levels of diseased and healthy animals did not reveal any significant variations on the 0^{th}, 30^{th} and 60^{th} day of treatment. Freeman *et al.* (2005) and Reimann *et al*. (2017) reported similar findings that there was no association between serum MDA levels in clinical stages of mitral valve disease in dogs. The mean value of lipid peroxides was high in dogs with valvular heart disease (4.93±0.64nM/mL) when compared to healthy animals (2.85±0.12nM/mL) on the initial day of treatment. According to Prasad *et al*. (1996), high level of MDA in cardiac tissues may exist during heart diseases and that need not be reflected in serum.

The MDA levels decreased to 2.93±0.74 from 4.93±0.64 nM / mL on 60^{th} day of treatment with coenzyme Q10 even though no statistical significance was observed In conditions like coronary artery disease, diabetes and hepatocellular carcinoma in humans, a reduction in the MDA levels have been recorded after coenzyme Q10 supplementation (Lee *et al.*, 2011; Moazen*et al.*, 2015; Liu *et al*., 2016). Litarru and Tiano (2007) reported that coenzyme Q10 had the ability to prevent the peroxidation of lipids present in cell membrane and circulation. A significant reduction in MDA levels in the coenzyme Q10 supplemented animals could be expected if administered for a longer period of time.

The effect of coenzyme Q10 supplementation on total antioxidant status and lipid peroxides are presented in the table below.

	Total antioxidant status (µM/L)			Lipid peroxides (nM/mL)		
	0th day	30th day	60th day	0th day	30th day	0th day
Diseased animals	1599.43±132.28^{aA}	1408.71±110.37^{aA}	910.17±118.17^{bB}	4.93±0.64	3.45±1.02	2.93±0.74
Healthy animals	824.78±75.29^{b}	824.78±75.29^{b}	824.78±75.29^{b}	2.85±0.12	2.85±0.12	2.85±0.12

Mean±S.E. bearing different small letter as superscripts differ significantly within column (between diseased and healthy animals) at 1% level (p≤0.01)

Mean±S.E. bearing different capital letter as superscripts differ significantly within rows for each parameter at 1% level (p≤0.01)

Based on the results of the present study, supplementation of coenzyme Q10 can be considered in the management of oxidative stress associated with chronic valvular heart disease in dogs. The quality of life of the patient could be improved with the administration of coenzyme Q10.

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Effect of dietary incorporation of ksheerabala residue on growth performance in wistar rats

Deepak Chandran[1], **K. Jasmine Rani**[2], **K.Shyama**[3] **and K.Ally**[4]
Department of Animal Nutrition, College of Veterinary and Animal Sciences,
Mannuthy – 680651, Thrissur, Kerala.
Kerala Veterinary and Animal Sciences University.

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Abstract

Ksheerabala residue is a by-product obtained during the preparation of ksheerabala oil which is made by incorporating bala (Sida cordifolia), cow milk and gingelly oil (Sesamum indicum). This residue is available in considerable quantity and many farmers use this byproduct for feeding livestock. But the level of incorporation and the effect of Ksheerabala residue on growth in rats are not yet well studied. Hence, the present study was planned to evaluate the effect of dietary incorporation of Ksheerabala residue as a feed resource in the diet of Wistar rats on their performance. Twenty-four male Wistar albino rats weighing 80 ± 5g were used as experimental animals and were allotted randomly to two treatments of six replicates each. Group 1 was fed basal diet as per BIS specification (control-T_1) and other group was fed a diet containing 5 per cent Ksheerabala residue (T2). The average body weight, body weight gain, dry matter intake, haematological and biochemical parameters were found to be similar in both the groups (P >0.05). The results of the present study indicate that Ksheerabala residue can be included in the rat ration up to five per cent level without any adverse effect on their growth performance.

Key words: *Ksheerabala residue, Rat, Blood, Growth*

In normal production systems, it has been traditional to feed conventional feeds notably cereals, oil cakes and meals to both ruminants and non-ruminants. But increases in feed ingredient prices and the scarcity of grains and plant protein supplements are the most important constraints hampering the animal production sector in India. Additionally, inadequate production of farm crops to meet the needs of both humans and their domestic animals had led to competition between man and livestock for these feed ingredients. This has forced animal nutritionists to intensify research into the feeding values of potentially useful, attractive, cheaper and non-conventional

1. *MVSc Scholar, Veterinary Biochemistry*
Email ID- drdeepakchandran24@gmail.com, Phone- 9400723398
2. *Assistant Professor*
3. *Associate Professor and Head, Cattle Breeding Farm Thumburmuzhy*
4. *Professor and Head*

feed resources (NCFR). Dafwang *et al.* (2001) reported that non-conventional feedstuffs could be considered as the best alternative to produce cheaper feed and ultimately lower the cost of meat and other animal products. Many of the NCFR which were designated as waste could be utilized and converted by animals into valuable products for human benefit to alleviate the problem of existing limited feed resources (Vasta *et al.,* 2008). Major constraints in the utilization of NCFR for animal feeding include high nutritional variability, low digestibility, seasonal production and presence of undesirable contaminants of organic or inorganic origin (Al-Masri, 2005; Weinberg *et al.,* 2008). Use of agro industrial by-products and crop residues in animal nutrition has been successfully adopted as a strategy to reduce feeding costs. But still a lacuna exists and the time has come to explore and utilize more unconventional feeds, even available in small quantities, so that they can be incorporated in the feeds of livestock and thus can reduce the competition between humans and livestock for common food grains.

The World Health Organization has encouraged the use of medicinal herbs and plants to minimize or substitute the use of chemicals through the global trend to go back to nature (Allam *et al.,* 1999) and utilization of medicinal plants as growth promoters in animal feeds has increased over the last few years due to prohibition of most of the antimicrobial growth promoters because of their residual effects (Acamovic and Brooker, 2005). Kerala, famous for Ayurveda, has various ayurvedic pharmaceuticals and by-products from these pharmaceuticals which are mainly composed of residues of medicinal herbs. Ksheerabala residue is an ayurvedic by-product obtained during the preparation of ksheerabala oil which is made by incorporating *Bala* (*Sida cordifolia* Linn., belongs to Malvaceae family), *Ksheera* (cow milk) and *Tila Taila* (Sesamum oil). This residue is available in considerable quantity and many farmers use this byproduct to feed domestic animals. But the feeding value of these residues as potential non-conventional feed resources are yet to be explored. Hence as a pilot study this research work was undertaken to evaluate the nutritive value of Ksheerabala residue and to assess the effect of dietary incorporation of Ksheerabala residue as a feed resource in the diet of Wistar rats on their performance.

Materials and Methods

Twenty four male Wistar albino rats weighing 80 ± 5g, selected from Small Animal Breeding Station, College of Veterinary and Animal Sciences, Mannuthy, formed the experimental subjects for the study. They were allotted randomly to two treatments of six replicates each for a period of one month. Rats belonging to group 1 were fed on a basal diet (T_1) (as per BIS specification) and animals of group 2 were fed on a diet (T_2) containing 5 per cent ksheerabala residue. Both the rations were made isonitrogenous and isocaloric. Proximate composition of Ksheerabala residue and ingredient and chemical composition of experimental rations were analysed. A weighed quantity of feed was given in the morning. Clean, fresh drinking water was provided *adlibitum* to all animals. Animals were maintained under normal ambient conditions. Data on quantities of feed offered daily were recorded. The left over portion of the feed was weighed daily and moisture content of feed given and balance feed were analyzed to calculate the daily dry matter intake. Body weights of all the rats were recorded at weekly intervals. Based on the body weight, feed requirements was reviewed fortnightly.

Blood samples were collected from six animals from each group at the end of the experiment. Haematological parameters were analysed with fresh blood samples using a haematological analyzer (Mythic 18 vet). Plasma total protein, plasma glucose (GOP-PAP methodology using standard kits), serum total cholesterol and serum triglycerides were determined by enzymatic colorimetric methods using standard kits supplied by Agappe diagnostics Ltd, Ernakulam, India. Alanine-aminotransferase (ALT) and aspartate-aminotransferase (AST) enzymes were measured according to the method described by Reitman and Frankel (1957).

Feed samples were analyzed for proximate principles (AOAC, 2016). Data were analyzed statistically using Analysis of Variance (Snedecor and Cochran, 1994).

Results and Discussion

The results obtained during the course of present study are discussed below.

Presented in Table 1 is the chemical composition of Ksheerabala residue. Ksheerabala residue contained 92.55 per cent dry matter, 29.52 per cent crude protein, 13.26 per cent ether extract, 6.39 per cent crude fibre, 8.42 per cent ash, 42.41 per cent nitrogen free extract, 0.06 per cent acid insoluble ash.

Data on the body weight of rats have been presented in the Table 2. There was no significant difference (P>0.05) in body weight between animals fed two dietary treatments. Total weight gain and average daily gain of the rats were similar in both the dietary treatments. These results were in agreement with Ogungbemi *et al.* (2017).

Total dry matter intake during the experimental period was 407.92 and 422.54 g respectively for animals fed T_1 and T_2. (Table 2) and statistical analysis of the data did not reveal any significant difference (P> 0.05) among the groups. Manjula *et al.* (2016) observed a similar total dry matter intake in rats. Additionally, rats fed with a diet containing 5 per cent Ksheerabala residue had almost similar weight gain and feed conversion efficiency as that of the control group.

Data on haematological and biochemical studies have been given in Table 3 and 4. The values of various haematological and biochemical parameters were similar in both groups indicating that dietary incorporation of Ksheerabala residue did not affect these parameters to any significant effect.

All the physiological and biochemical parameters recorded in the present study fell in the normal range reported for the species. Akpanabiatu *et al*. (2003), in a study of the biochemical effect of *Eleophorbia drupifera,* reported a decreased AST level in treated groups. Similar changes in ALT and AST activities in animals treated with plant extracts were reported by Bumah *et al*. (2005).

Table 1: Chemical composition of Ksheerabala residue and experimental feeds

Parameter	Ksheerabala Residue	T_1	T_2
Dry matter	92.55	91.90	91.98
Crude Protein	29.52	24.38	24.72
Ether extract	13.26	5.23	5.28
Crude fibre	6.39	5.26	5.78
Ash	8.42	6.77	7.85
Nitrogen free extract	42.41	58.36	56.37
Acid insoluble ash	0.06	0.82	0.78

Table 2: Body weight (g) and dry matter intake (g) of experimental rats

Parameters	T_1	T_2
Initial body weight (g)	85.62± 2.82	84.66 ± 3.83
Final body weight (g)	132.56 ± 8.90	137.33 ± 9.07
Total weight gain (g)	46.94 ± 3.52	52.67 ± 4.52
Total dry matter intake (g/animal)	407.92 ± 21.34	422.54 ± 22.16

T_1 and T_2- mean of six values. (P > 0.05).

Table 3: Haematological parameters of experimental rats

Parameters	T_1	T_2
Haemoglobin (mg/dl)	14.78 ± 2.60	14.52 ±0.34
MCV (fL)	58.04 ± 0.56	58.29 ± 1.20
MCH (pg)	18.20 ±0.43	18.85 ± 0.39
MCHC (g/dl)	34.15 ± 0.39	33.28 ± 0.57
RBC count (x 10^6/µl)	8.84 ±0.51	8.08 ±0.32
WBC count (x10^3/µl)	10.32 ±0.14	10.44 ± 0.37

T_1 and T_2- mean of six values. (P > 0. 05).

Table 4: Blood biochemical parameters of experimental rats

Parameter	T_1	T_2
Albumin, (g/dl)	3.42 ± 0.14	3.88 ± 0.20
Plasma protein, (g/dl)	6.30 ± 0.05	6.33 ± 0.06
Calcium, (mg/dl)	11.23 ± 0.11	11.26 ± 0.23
Phosphorus, (mg/dl)	7.04 ± 0.10	7.05 ± 0.11
Cholesterol, mg/dl	78.34 ± 2.41	74.88 ± 2.63
Triglyceride, (mg/dl)	44.88 ± 5.89	38.33 ± 4.88
Glucose, (mg/dl)	89.78 ± 1.26	87.19 ± 1.71
AST(u/l)	105 ± 8.26	101 ± 9.23
AST(u/l)	21.52 ± 3.24	19.62 ± 2.96

T_1 and T_2- mean of six values. (P > 0.05).

On summarizing the overall effects of the study, it could be inferred that Ksheerabala residue contains high CP, EE and CF value and can be included in the rat ration up to a level of 5 per cent without any adverse effect on their growth performance.

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RESEARCH ARTICLE
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In vitro antimicrobial activity of lemongrass *(Cymbopogon flexuosus)* oil and citral against Methicillin Resistant *Staphylococcus Aureus* (MRSA) from bovine mastitis*

S. Jisha[1], R.S. Suja[2], J. K. Bibu[2], S. Surya[2], R. Ambily[2], J. Reni[3], Thresia[4], V.K. Vidya[5], P. Gayathri[5] and P. T.A. Usha[6]

Department of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala. Kerala Veterinary and Animal Sciences University, Pookode, Kerala.

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Abstract

The mounting tide of bovine mastitis by methicillin-resistant Staphylococcus aureus (MRSA) poses serious threat to efficacy of antibiotic treatment. Therefore, plant derived essential oils and their constituents are gaining great importance in the recent times as promising antimicrobial agents. Hence the present study was conducted to evaluate antibacterial activity of lemongrass oil (LGO) and its active principle, citral against methicillin and oxacillin resistant Staphylococcus aureus (MRSA) isolated from bovine mastitis cases. Among the 16 S. aureus organisms isolated, six isolates identified as MRSA by antibiogram against methicillin and oxacillin were selected for further study. The findings of the study indicated significant antimicrobial activity of LGO and citral as evident from their minimum inhibitory concentration (MIC) and diameter of zone of inhibition values.

Keywords : *MRSA, LGO, Antibiogram, MIC*

Mastitis, a multi-etiological composite disease defined as the inflammation of parenchyma of mammary glands is characterised by pathological alterations in glandular tissues in conjunction with physical, chemical and bacteriological changes in milk (Radostits *et al*., 2000). It is a worldwide crisis as it adversely impinges, not only the milk quality and welfare of the animal, but also engenders enormous financial losses to every country; including developed ones by the substantial descend in milk production (Sharma, 2007). According to Guha and Gera. (2011), the incidence of mastitis is a consequence of interplay between three key aspects such as host resistance, infectious agents and environmental factors.

Staphylococcus spp. bacteria are one of the prime causes of clinical and subclinical

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1. *MVSc scholar and corresponding author (Email : jisha792@gmail.com, Ph: 9645046956)*
2. *Assistant Professor*
3. *MVSc Scholar*
4. *MSc Applied Microbiology Student, Department of Veterinary Microbiology*
5. *MVSc Scholars, Department of Animal Reproduction, Gynecology and Obstetrics*
6. *Professor and Head*

mastitis in dairy cattle, and the animals affected with subclinical mastitis in the herd when left untreated, may act as carriers of the pathogenic bacteria and can transmit the infection to other cattle as well as susceptible people. The widespread use of beta lactam antibiotics against bovine mastitis has led to the development of livestock-associated methicillin resistant *Staphylococcus aureus* (LA-MRSA) in cattle. LA-MRSA is mostly associated with multidrug resistance and biofilm formation leading to the persistent infections which are difficult to cure. In recent times, LA-MRSA infections been recognized as a rapidly evolving cause of human infections that may end in fatality.

The combination therapy, especially combining essential oil with the conventional antibiotics represents a vital strategy to ameliorate antimicrobial resistance. Essential oils (EOs) are the aromatic derivatives of plants that are used for centuries to treat infections. Recently these ancient remedies are being continuously explored as a new source of antimicrobial agents as they are active against a wide range of organisms. This is mainly attributed to the complex chemical constituents of these EOs which produces its action through the inhibition or interaction of multiple targets of cells (Boire *et al*., 2013). Moreover, the lipophilic nature of EOs makes it more active as this enables easy penetration into the bacterial cell (Kalemba and Kunicka, 2003).

Materials and methods

Microorganisms

The test microorganisms used in the study included 16 *S.aureus* strains isolated from clinical or subclinical mastitis, presented at Teaching Veterinary Clinical Complex, Mannuthy as well as from University Livestock Farm, Mannuthy. The working cultures of the bacteria were maintained on Brain Heart Infusion (BHI) agar, sub-culturing done weekly for a maximum of three weeks, to maintain viability and colony characteristics.

Essential oil and components

The EOs used in this study were lemongrass (*Cymbopogon flexuosus*) oil procured from Synthite Industries Pvt. Ltd., Kerala and citral, 95 % (C83007) purchased from M/s Sigma Aldrich, India. They were kept tightly closed in a dry ventilated area, protected from light.

Identification of methicillin resistant S. aureus (MRSA)

Antimicrobial susceptibility testing of the *S. aureus* isolates against methicillin (5 µg) and oxacillin (1 µg) was done for the identification of MRSA isolates by Kirby-Bauer disc diffusion method (Bauer *et al*., 1966) as per the Clinical and Laboratory Standards Institute guidelines.

Kirby-Bauer disc diffusion method

A sterile cotton swab on a wooden applicator was dipped into the standardised inoculum. Excess fluid was removed by pressing and rotating the swab against the side of the tube above the suspension. The entire agar surface of the Mueller Hinton agar (MHA) plate was streaked with the swab three times, turning the plate at 60° angle between each streaking to ensure even distribution. The methicillin, oxacillin and cefoxitin discs were placed equidistant from each other on the agar surface and the plates were incubated at 35°C for 18 to 24 h. After incubation, the zone of inhibition was measured and interpreted as either susceptible or resistant to the exposed agent according to Clinical Laboratory Standards Institute criteria. Accordingly, diameter of inhibition zones of ≤10, 11-12, and ≥13 mm was categorized as resistant (R), intermediate (I), or susceptible (S) to methicillin and oxacillin. For cefoxitin disc, a diameter of inhibition zones of ≤21 mm and ≥22 mm to the Staphylococci was considered as resistant and susceptible respectively (CLSI, 2016).

Antimicrobial susceptibility of MRSA against LGO and citral

Disc diffusion assay and Minimum Inhibitory Concentration determination methods were employed to assess the antimicrobial susceptibility of the MRSA isolates against LGO and its primary chemical component citral as per the procedure described by De silva *et al*. (2017) with minor modifications.

Disc Diffusion Assay

Under aseptic conditions, five 6 mm diameter sterile discs were impregnated with 10 µL of different dilutions of LGO/citral in 5 per cent Tween 20 at 1:1, 1:2, 1:5 and 1:10 ratio *i.e.*1 part of the LGO or citral in respective parts of 5 per cent Tween 20 (vehicle) and were placed symmetrically by means of sterile disc holding forceps on the surface of agar plates. One of the discs was moistened with 5 per cent tween 20 served as vehicle control. All Petri dishes were sealed with sterile laboratory parafilm to avoid eventual evaporation of the test samples. The plates were incubated at 35ºC for 24 h and the diameter of the zones of complete inhibition measured as >10 mm was considered significantly inhibitory as denoted by Fu *et al*. (2007).

Determination of Minimum Inhibitory Concentration (MIC)

For the measurement of MIC by microbroth dilution method, a modified resazurin microtitre plate assay was employed as reported by Elshikh *et al*. 2016 with minor modifications. An aliquot (50 µL of Cationated Mueller Hinton Broth (CAMHB) supplemented with 2% NaCl, containing 0.5 % (v/v) Tween 20 was added to wells of a sterile 96-well microtitre plate. Two fold serial dilutions of LGO and citral were made by using a multi-channel pipette, for which 50 µL of test substance was added initially in the first well followed by sequential transferring of 50 µL to the subsequent wells after proper mixing, and finally, 50 µL from the last well was discarded. Next, 50 µL of bacterial suspension was added to each well to achieve a concentration of approximately 5×10^5 CFU/mL in the well. The final concentrations of LGO/citral obtained in the wells were 4, 2, 1, 0.5, 0.25, 0.125, 0.0625, 0.0312, 0.0156, 0.0078, 0.0039 and 0.0019 % v/v. The growth control (inoculum/positive control) wells contained 50 µL each of CAMHB medium and bacterial cells without test substances (LGO/citral) while the sterility control (media/vehicle/negative control) wells contained 100 µL CAMHB only. The plates were incubated at 35°C for 24 h and subsequently 30 µL of 0.01 per cent resazurin was added in each well, mixed by gentle shaking and the plates were again incubated at 37°C for 3 h. Bacterial growth was monitored visually as colour change from blue to pink, which indicated the presence of viable cells in cultures (Elshikh *et al*., 2016). The MIC was defined as the lowest concentration at which visible growth was inhibited *i.e.,* the lowest concentration that remained blue in colour as the colour change from blue to pink was inhibited (Sanchez *et al.,* 2016). Each test reaction was done in triplicates.

Results and Discussion

Identification of methicillin resistant S. aureus (MRSA)

A total of 16 *S. aureus* isolates were tested for methicillin/ oxacillin resistance by Kirby-Bauer disc diffusion test. Out of the 16 strains, six isolates showed resistance against both methicillin and oxacillin and were identified as methicillin resistant *S. aureus.*

Antimicrobial susceptibility of MRSA against LGO and citral

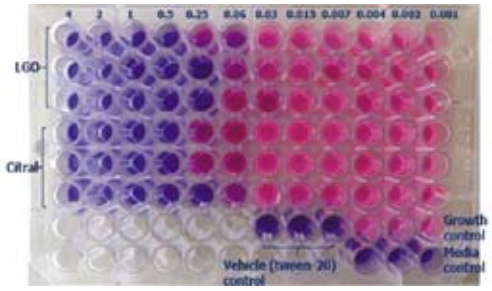
Disc Diffusion Assay

The diameter of zone of inhibition varied from 19.29 ± 1.2 to 9.51 ± 0.38 mm (table 1). The vehicle control disc coated with 5% tween-20 in sterile water did not show any inhibitory zone. Both LGO and citral exhibited significant inhibition zone (>10 mm) against MRSA isolates at all dilutions except for citral at 1:10 ratio. LGO at 1:1 ratio was found to be most effective amongst all the treatment groups with a mean inhibition zone of 19.29 mm followed by citral at 1:1 ratio (18 mm). Moreover, a dose dependent decrease in the diameter of zone of inhibition was noticed for both lemongrass oil and citral.

Determination of Minimum Inhibitory Concentration (MIC)

The vehicle control wells containing 0.5% tween-20 did not show any inhibition, whereas LGO and citral showed MIC values of 0.33 % v/v and 0.67% v/v respectively, indicating that LGO possessed higher activity than citral. Besides, the increase in the sensitivity of

Fig. 1. Determination of MIC of LGO and citral



MRSA isolates towards LGO was found to be significant ($p< 0.01$) as compared to the citral treatment group.

In the study, disc diffusion assay was performed against the MRSA isolates at various dilutions of LGO and citral in 5% tween 20 (1:1, 1:2, 1:5, and 1:10 ratio). The tween 20 used in the experiment serves as a solubilising agent that prevent the possible unequal distribution of the oils through the medium, without interfering bacterial growth and differentiation. Unlike Tween-80 and DMSO, it has little or no effect on the activity of the essential oils. In this method activity of the oils were compared using zone of inhibition around the disc usually expressed in diameter (Kalemba and Kanuka, 2003). Results indicated that both LGO and citral possess significant antimicrobial activity against MRSA with a zone of inhibition greater than 10mm except citral at 1:10 (Fu *et al*., 2007), while the disc coated with 5% tween-20 in sterile water (vehicle control) did not show any inhibitory zone. The maximum zone of inhibition obtained for LGO and citral were 19.29 and 18 mm respectively. Moreover a dose dependent decrease in the diameter of zone of inhibition was noticed for both lemongrass oil and citral.

The MIC values obtained for the LGO using modified resazurin microtitre plate assay against MRSA isolates was significantly higher than that of citral, which could be attributed to the presence of other components of LGO that had antimicrobial activity. This was in consensus with the study conducted by Aiemsaard *et al*. (2012), in which LGO obtained higher inhibition zone when compared to the citral. The higher antimicrobial activity of LGO over citral could be either attributed to the direct antimicrobial effects produced by the major components such as geraniol or to the synergism arising from the potentiating action of minor components (linalool, geraniol acetate and myrcene) of LGO (Aiemsaard *et al*., 2011). This was also regarded as one of the reason for the additive and synergistic effects produced by the combination of these essential oils *C. citratus* with *C. giganteus* (Bassole *et al.*, 2011). However, the LGO elicits the antimicrobial effects by producing distortion of cytoplasmic membrane and swelling of cell wall, whereas citral affects the stability of bacterial cell membrane causing cell leakage (Aiemsaard *et al*., 2011 and Taufik *et al*., 2017). On contrary, there are studies that have obtained same MIC values for both LGO and citral suggesting that the whole essential oils are more suitable than the pure compounds whose isolation is difficult and costly practice in the drug development (Onawunmi, 1989; Christensen and Anderson, 2017).

Table.1 Diameter of zone inhibition of LGO and citral against MRSA isolates, mm

Dilutions	Zone of inhibition (mm)	
	LGO	Citral
1:1	19.29 ± 1.2^{b}	18.0 ± 0.76^{d}
1:2	17.13 ± 1.12^{ab}	15.71 ± 0.79^{c}
1:5	15.25 ± 1.46^{ab}	13.37 ± 0.74^{b}
1:10	13.58 ± 1.42^{aA}	9.51 ± 0.38^{aB}

Values are expressed as Mean ± SE, n=6: Values bearing different superscripts (a-b in column and A-B in row) vary significantly at $p< 0.05$. No inhibition zone was observed with vehicle control; Diameter values of zone inhibition are inclusive of disc diameter of 6 mm.

Table. 2 Minimum inhibitory concentration of lemongrass oil and citral against MRSA isolates, % v/v

Isolate ID	Lemongrass oil	Citral
SA1	0.25	0.5
SA2	0.25	0.5
SA3	0.5	1
SA4	0.25	0.5
SA5	0.25	0.5
SA6	0.5	1
Mean ± SE	**0.33 ± 0.53^{a}**	**0.67 ± 0.10^{b}**

Values of MIC are mean of triplicates, n=6; Values bearing different superscripts vary significantly at $p< 0.05$.

Thus, it could be concluded that the lemongrass oil extracted from *Cymbopogon flexuosus* has marked antimicrobial activity against methicillin resistant *S. aureus* strains isolated from bovine mastitis.

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RESEARCH ARTICLE
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Influence of thermal stress on feed intake and body condition score during early postpartum period of crossbred cows in Kerala*

C. Ibraheem Kutty[1], C. P. Abdul Azeez[2], K. Promod[3], B. Bibin Becha[4], C. Sunanda[5], S. Lasna[6] and K.S. Anil[7].
Livestock Research Station, Kerala Veterinary and Animal Sciences University
Thiruvazhamkunnu P.O., Palakkad District, Kerala Pin code 678601

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Abstract

Increased milk production and environmental alterations caused by climate change makes dairy animals highly vulnerable to Thermal Stress (TS). Cross bred cattle shows some level of adaptation, characterized by reproductive performance of low level, almost uniformly throughout the year. Hence, objective of the present study was to find out the pattern of feed intake and body condition score of cross bred cows in the context of adaptation to TS across seasons prevailing in Kerala.

The study was carried out at Livestock Research Station, Thiruvazhamkunnu, Kerala, in 22 post partum cows between Day 7 and Day 135 of calving. Daily intake of concentrate and weekly body condition score (BCS) were recorded. Blood samples were collected from 10 cows every week for estimation of thermal stress indicators and the climatic variables were recorded daily. Data were analyzed for monthly and seasonal variations and correlations between each other.

Temperature Humidity index (THI) and other climatic variables showed exposure of the animals to TS throughout the year. During summer months, ambient temperature and THI were significantly higher to cause moderate TS. Feed intake, BCS and cortisol levels were significantly high during summer, even though there was no significant correlation with climatic stress factors. HSP 70 was elevated throughout the year and significantly high during the period of moderate THI, indicating adaptation to TS. It is concluded that higher feed intake and BCS during summer months is attributable to TS adaptation because of continuous exposure across many years.

Keywords: *Thermal stress; Adaptation; Climate, Season; Feed intake; BCS*

** Forms part of the PhD thesis of the first author submitted to Kerala Veterinary and Animal Sciences University, Pookode, Wayanad, Kerala*

1. *Corresponding author: C Ibraheem Kutty, PhD scholar, Department ARGO, College of Veterinary & Animal Sciences, Pookode, Wayanad Dt., Kerala. Mob: 9562497320, ibraheemkutty50@gmail.com*
2. *Assistant Professor, Dept. of ARGO., CVAS Pookode*
3. *Associate Professor, Dept. of ARGO., CVAS Pookode*
4. *Assistant Professor, Dept. of ARGO., CVAS Mannuthy*
5. *Assistant Professor, Department of Statistics, CVAS Pookode*
6. *Assistant Professor, Livestock Research station, Thiruvazhamkunnu*
7. *Professor and Head, Department of LPM, CVAS, Mannuthy*

Milk production of dairy cows has been increasing over the years, achieved through planned improvements in management as well as genetic potential. The progress in milk production is associated with an increase of feed intake (Cain *et al*., 2006) and high metabolic heat increment, which predisposes the animal to thermal stress (TS). Simultaneously, environmental alterations caused by climate change phenomena seriously affect the animal unless thermoregulatory mechanisms are highly efficient (Schuller *et al.,* 2017).

Thermal stress has been found to reduce the voluntary feed intake as a natural mechanism to reduce the metabolic heat production (Allen *et al.,* 2009), so that dairy cows are in a state of negative energy balance (Guo *et al*., 2018) and this affects lactation and body condition. In this respect, body condition score (BCS) is a simple tool for assessing the energy status of animals especially dairy cattle (Morris *et al.,* 2011; Smijisha, 2012).

In tropical and subtropical regions, high ambient temperature (AT) forms the major reason for TS, and the temperature humidity index (THI) is often used as a composite measure of TS. While high yielding and pure-bred cows are seriously affected, cross bred dairy cattle in Kerala has been found to have varying levels of adaptation against TS (Prasad, 2014), reflected by more or less uniform reproductive performance throughout the year in spite of continuous exposure to TS (Kutty *et al.*, 2019). In this context, present study was carried out to find out the pattern of feed intake and body condition score of cross bred dairy cows reared under mild to moderate levels of TS across various seasons in Kerala.

Materials and methods

The study was carried out at Livestock Research Station, Thiruvazhamkunnu under Kerala Veterinary and Animal Sciences University. The dairy farm of the station was having around 300 heads of cross bred dairy cattle managed intensively as per the standard recommendations (ICAR-NIANP, 2013). The study involved observations on 22 cows between Day 7 and Day 135 postpartum for a period of one year from September 2018 onwards. Every month, 6-8 cows of the study group were replaced with newly calved ones conforming to the study requirements.

The study animals were fed with green grass and concentrate pellets based on feed computation revised every week considering the requirements of maintenance and milk production. Feeding was done in individual troughs to avoid wastage and cross feeding. The quantity of concentrate fed on daily basis and body condition score (BCS) at weekly intervals for each animal was recorded. BCS recording was done subjectively as suggested for cross bred cattle by Smijisha (2012) using 1 to 5 scale with increments of 0.5. The animals were observed from behind and sides of the body focusing changes at seven prominent parts, and a composite score was obtained taking average of all the observations.

Blood samples were collected at weekly intervals from 10 cows of the study group selected randomly, serum was separated and stored frozen for estimation of stress indicators in serum such as HSP 70 and cortisol using ELISA kits (Neogen, USA). Daily ambient conditions were recorded using data logger (HOBO pro V2), installed within the barn and hourly recordings of ambient temperature (AT) and relative humidity (RH) were done. Climatic parameters such as maximum (Mx), mean (Av) and minimum (Mn) of AT and RH were collected and THI values were calculated using the formula (LPHSI, 1990).

$$\text{THI } (LPHSI) = T - \left[\left(0.55 - \frac{0.55 \times RH}{100}\right) \times T - 58\right]$$

Where T - Average temperature (in Degree Fahrenheit)

RH - Per cent relative humidity

The period of study was divided into four seasons of three months each based on the pattern of rainfall and day length (Kutty *et al.,* 2019) such as North East monsoon (SON), Post monsoon (DJF), Summer (MAM) and South West monsoon (JJA) comprised of months such as September to November, December to February, March to May and June to August

Table 1. Monthly averages of ambient temperature, relative humidity, THI, feed intake and body condition score

Month	Ambient Temperature (°C)	Relative Humidity (%)	THI	Feeding (Kg)	BCS
Sep	28.35	83.84	80.81	6.29	2.84
Oct	28.64	79.08	80.62	6.42	2.90
Nov	29.00	73.78	80.42	6.32	2.90
Dec	28.55	72.39	79.54	6.16	2.99
Jan	26.78	62.85	75.67	8.38	3.03
Feb	29.89	63.43	80.21	7.44	3.19
Mar	31.48	64.45	82.67	8.42	3.10
Apr	32.02	69.22	84.28	7.96	3.16
May	29.86	79.86	82.68	8.48	3.15
Jun	29.12	81.69	81.75	7.92	3.10
Jul	27.35	86.18	79.47	7.53	2.97
Aug	26.89	88.02	78.93	6.75	3.08
Mean	29.00	75.40	80.59	7.34	3.03

Table 2. Quarterly averages of climatic variables and stress indicators in the serum

Quarter	Av. Temp (°C)	Av. RH (%)	THI	MnT (°C)	MxT (°C)	HSP70 (ng/ml)	Cortisol (ng/ml)
SON	28.67±0.19	78.90±2.91	80.62±0.11	24.25±0.34	33.09±0.70	1.97±0.20	7.12±1.46
DJF	28.41±0.90	66.22±3.09	78.47±1.41	22.10±.19	34.72±0.86	2.61±0.07	6.73±1.04
MAM	31.12±0.65	71.18±4.56	83.21±0.53	26.31±0.66	35.93±1.84	6.20±0.44	9.36±1.28
JJA	27.79±0.68	85.30±1.88	80.05±0.87	24.76±0.38	30.82±0.98	3.01±0.19	7.92±1.56
Total	29.00±0.47*	75.40±2.60*	80.59±0.63*	24.35±0.55*	33.64±0.77^{ns}	3.45±0.50**	7.78±0.65**

**. Significant at 5% ** Significant at 1% ns – non significant*

Table 3. Comparison of feed intake (in Kg) during the four seasons of study

Season	Feed intake (Kg)	BCS (Raw)	BCS for Lactation stages
SON	6.34 ± 0.04^{a}	2.87 ± 0.01^{a}	3.10 ± 0.00
DJF	7.32 ± 0.64^{ab}	3.07 ± 0.06^{b}	3.00 ± 0.07
MAM	8.28 ± 0.16 ^{b}	3.17 ± 0.01^{b}	3.10 ± 0.04
JJA	7.40 ± 0.34 ^{ab}	3.05 ± 0.04^{b}	3.05 ± 0.03
Overall	7.33 ± 0.26	3.03 ± 0.03	3.06 ± 0.02
F value	4.50*	7.724**;	0.408^{ns};
p- value	0.039	0.010	0.748

**. Significant at 5% ** Significant at 1% ns – non significant*

Values with different superscript varies significantly within column

respectively. The climatic variables, stress indicators and other study parameters were analyzed for descriptive details, monthly and seasonal variations and correlation between study parameters using SPSS software (SPSS V. 24.0.).

Results and discussion

Yearly averages of AT, RH and THI were 29.00ºC, 75.40 per cent and 80.59 respectively indicating moderate climate during the study period. Monthly averages of AT, RH, THI, feed intake and BCS are shown in Table 1. AT ranged from 26.78 ºC in January to 32.02 ºC in April. Since the lowest monthly mean of AT exceeded 26ºC, it can be inferred that climate of the locality cannot be designated as winter in any of the months as opined by Rao (2013). Hence, the quarter comprising lowest monthly

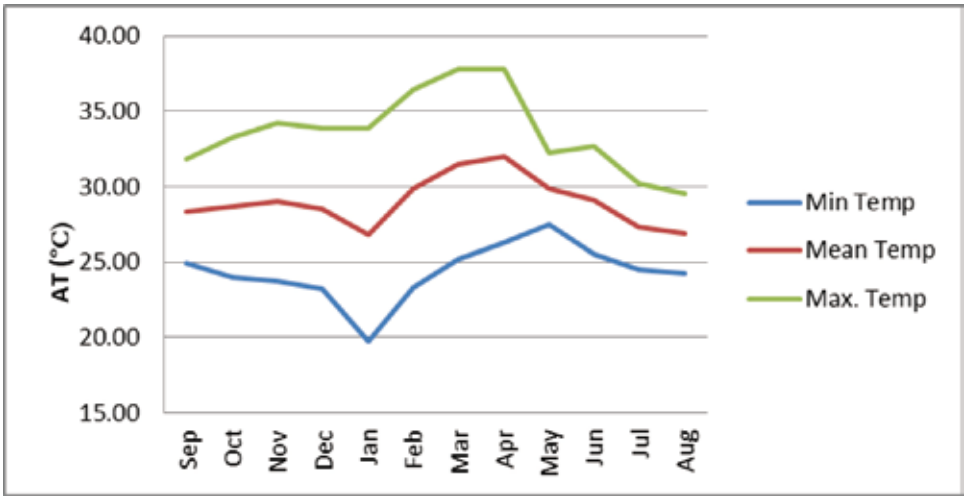

Fig. 1. Monthly trends of daily mean, minimum and maximum ambient temperatures

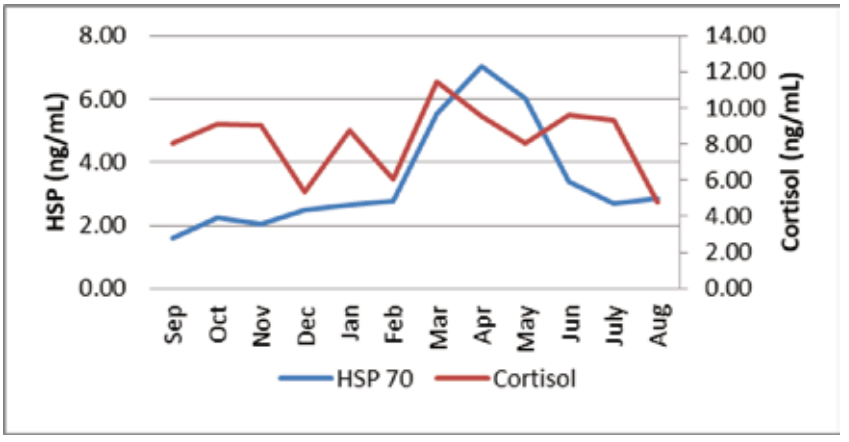

Fig.2. Monthly trends of HSP 70 and Cortisol during the study period

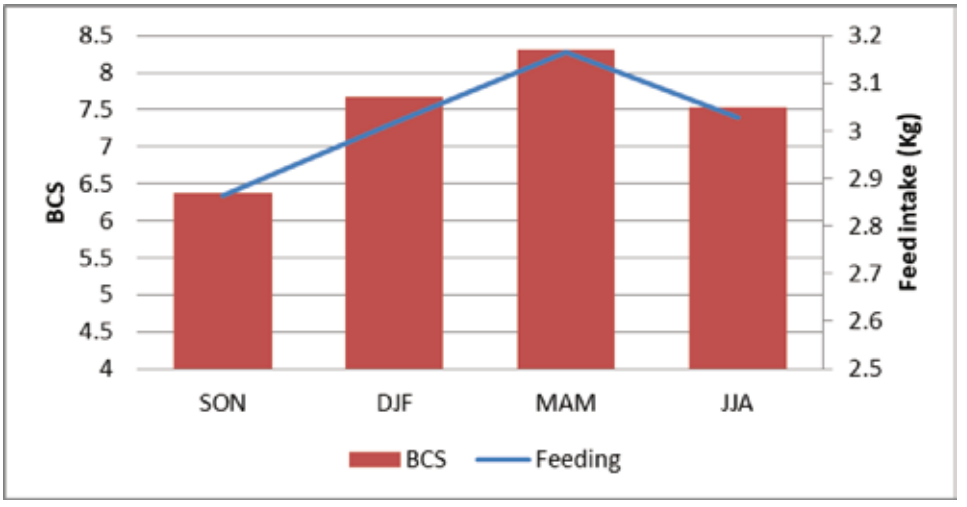

Fig. 3. Comparison of BCS and feed intake across the four seasons

mean of AT is designated as post monsoon in the study.

Annual trends based on monthly averages of Mn, Av and Mx AT of the study locality are shown in Fig. 1. Corresponding to the lowest monthly value for daily mean AT in January, MnT and MxT lines showed a depression in January, while the highest monthly means of MnT and MxT varied slightly from that of daily AvT. The variations of Mn AT, Av AT and Mx AT between months were highly significant indicating considerable variation of climate between months causing more stress to the animals (Krishnan *et al*., 2017).

Changes in RH corresponded to the amount of rainfall received with highest during August and lowest during January in the study year. THI was beyond 75 throughout the year, indicating mild to moderate stress exposure throughout the year and the yearly average was more than 80, which forms the threshold level for moderate TS (Das *et al*., 2016). Similarly, the highest THI was during April (84.28) indicating that none of months had THI level leading to severe stress. Even though RH also contributes to TS by affecting heat dissipation from the animal body, monthly means of THI showed almost similar pattern as that of AT across months, which indicates that elevation of temperature is the major contributor of TS.

Quarterly averages of daily mean AT, RH, THI, Mn AT and Mx AT and stress indicators such as HSP70 and cortisol are shown in Table 2. Corresponding to the highest value of AT and THI during summer, both HSP 70 and cortisol levels were also highest during the period with highly significant variation between quarters, which indicated that the study animals were affected by TS. While HSP 70 had the same pattern of variation across seasons comparable to that of AT and THI, the pattern of variation was different for cortisol. This indicated that HSP 70 was more specific for TS, whereas cortisol indicated combined influence of other stress factors as well affecting the animal.

Monthly trends of HSP 70 and cortisol are shown in Fig. 2. Overall mean value of HSP 70 throughout the study period (3.45 ng/ml) exceeded the level (1.5 to 2.0 ng/ml) prescribed for animals not exposed to TS (Archana *et al*., 2017), indicating that the animals were under TS during most part of the year. While, levels of HSP 70 showed consistent variation between months, levels of cortisol was highly variable, indicating involvement of different factors in the regulation of these stress indicators (Rajoriya *et al.,*2014).

Monthly average of feed intake is also shown in Table 1. The intake was more from January to July, with the highest values during March to May – the period of maximum TS. Since the animals were not fed *ad libitum*, and the feed intake represents the quantity offered based on computation for the milk yield as well, it can be inferred that the yield of animals in summer was more, necessitating increased feed allocation during summer months. Thus, highest feed intake coincided ($P<0.05$) with the highest AT and THI of summer, which is contrary to many reports that TS causes reduction of feed intake as a biological measure to reduce metabolic heat increment (Rhoads *et al.,*2009; Settivari *et al.,* 2007).

Feed intake and BCS of the study animals are compared across seasons in Table 3. BCS was significantly low during SON compared to other three seasons. The periods of highest and lowest, and the pattern of variations were similar for feed intake and BCS as shown in Fig. 3; so that BCS variations across seasons can be attributed to the feed intake. At the same time, feed intake did not show significant correlation with major climatic variables indicating lesser influence of TS, concurring to the report of Sonmez *et al.* (2005).

Since increased milk production affects body condition, mean BCS across seasons for each stage of lactation are also shown in Table 3. While raw BCS was significantly low during SON, BCS adjusted for lactation did not show significant variation between lactation stages or seasons. This implies that whatever difference in BCS noticed between seasons was due to change in feed intake. However, indirect influence of lactation on BCS was evident since feed allocation was

primarily based on milk yield, even though variation of BCS between lactation groups was non-significant (F-value= 0.22, p-value=0.344).

There was significant positive correlation between feed intake and BCS ($P<0.05$) as reported by Gaughan *et al.* (2013). HSP 70 also had the same pattern of variation across seasons with major elevation from February to June corresponding to the elevation of THI beyond 81. There was significant positive correlation ($P<0.01$) of HSP 70 with major climatic stress factors such as THI and AT. Since HSP 70 functions as molecular chaperones facilitating repair of cellular damage caused by TS, higher level of HSP 70 in the study forms an indication of adaptation to TS as reported by Archana *et al.* (2017).

The higher intake of feed and better BCS during summer and similar pattern of HSP 70 having significant correlation between each other indicated increased feed intake as against a decrease reported in many studies (Settivari *et al.,* 2007; Rhoads *et al.,* 2009), and was made possible by the adaptation to TS. Thus, significantly higher ($P<0.05$) feed intake during summer could be attributed to the tolerance of the animals against moderate TS. An increase of 7 to 25 per cent in maintenance requirement of cows during summer has been reported by Allen *et al.* (2009), even though severe TS was found to cause lactating cows to enter a period of negative energy balance (Rhoads *et al.,* 2009; Settivari *et al.,* 2007).

To conclude, the study animals were exposed continuously to TS indicated by THI value exceeding 75 throughout the year. During summer months, climatic variables were high enough to cause moderate TS, also reflected by elevation of HSP 70 and cortisol. However, feed intake and BCS were high during summer and no significant correlation was evident with major climatic stress factors. Hence, better feed intake and BCS during summer months was attributed to adaptation of these animals developed through continuous exposure to TS across many years and was reflected by the increased level of HSP 70 throughout the year and significantly higher levels during the period of moderately high THI.

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Effect of dietary incorporation of ksheerabala residue on nutrient digestibility and blood biochemical profile in Malabari kids*

T.J. Roshma[1], K. Ally[2], Thirupathy Venkatachalapathy[3], K. Shyama[4] and K. George Sherin[5].
Department of Animal Nutrition, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala
Kerala Veterinary and Animal Sciences University

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Abstract

An experiment was conducted with eighteen weaned Malabari kids of three months of age for 90 days to assess the effect of dietary incorporation of Ksheerabala residue on nutrient digestibility and blood biochemical profile in goats. Kids were divided into three groups (T_1, T_2 and T_3) as uniformly as possible with regard to age, sex and body weight and were offered kid starter containing Ksheearabala residue at 0, 10 and 20 per cent, respectively. Kid starters were made isonitrogenous and isocaloric (24 per cent CP and 70 per cent TDN). Kids were fed as per ICAR standards (Ranjhan, 1998). Green grass was offered as the sole source of roughage. Data on digestibility of nutrients and haematologcal parameters like haemoglobin, plasma protein, serum calcium, serum phosphorus, serum cholesterol and triglycerides were the criteria employed for evaluation and they did not show any significant difference (P >0.05) among the groups. It could be inferred that digestibility of nutrients and haematological parameters of kids were not influenced by the inclusion of Ksheerabala residue in kid starter.

Key words: Blood parameters, Digestibility, kid starter and Ksheerabala residue

Kerala has various ayurvedic pharmaceuticals and byproducts composed of residues of medicinal herbs. Ksheerabala residue is a byproduct obtained during the preparation of ksheerabala oil which is made by incorporating Sida cordifolia, cow milk and gingelly oil. This residue is available in considerable quantity locally, around 800-1000 kg per week (Kottakkal Aryavydiasala , kottakkal, Kerala) and many of the farmers are using for feeding goats. The feeding value of these residues as potential non conventional feed resource (NCFR) are yet to be explored. The main problem

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Correspondence E-mail: ally@kvasu.ac.in Mob:9447476508

1. *Veterinary Surgeon, Animal Husbandry Department, Kerala*
2. *Professor and Head*
3. *Professor and Head, University Sheep and Goat Farm, Mannuthy*
4. *Professor and Head, Cattle Breeding Farm, Thumburmuzhi*
5. *Assistant Professor, Base Farm, Kolahalamedu*

with residues from ayurvedic products are their impalatability and probable cumulative toxic effects. The microflora of goats' rumen can utilize the fibrous residues in the byproducts of ayurvedic preparations and can possibly detoxify the intrinsic factors present in these residues to some extend (Abreu et al.2004) Hence the present study is planned to evaluate the effect of dietary incorporation of Ksheerabala residue as a NCFR in the diet of kids on nutrient digestibility and blood biochemical profile.

Materials and Methods

Eighteen healthy kids of three months of age around 8.25 kg body weight , selected from University Goat and Sheep Farm, College of Veterinary and Animal Sciences, Mannuthy, formed the experimental subjects for the study. Kids were weaned and housed individually in well ventilated, clean and dry shed with facilities for feeding and watering. They were divided into three groups of six animals each as uniformly as possible with regard to age, sex and body weight and were allotted randomly to one of the three groups, T_1 (kid starter) , T_2 (kid starter containing 10 per cent ksheerabala residue) and T_3 (kid starter containing 20 per cent ksheerabala residue). All the rations were made isonitrogenous and isocaloric (24 per cent CP and 70 per cent TDN). Good quality green grass and clean drinking water were offered from first week of age. Ksheerabala residue available after extraction of oil from the pharmaceutical firm (Kottakkal Aryavydiasala, Kerala) was procured freshly as a firm solid block of around 35-40 kg weight. Proximate composition of Ksheerabala residue is presented in Table 1.The ingredient and chemical composition of experimental rations are presented in Table 2.

The experimental animals were maintained on the respective treatments for a period of 90 days. Daily records of concentrate and grass given and balance if any were maintained throughout the feeding trial. Data on daily dry matter intake was maintained throughout the experimental period. A digestibility trial involving five days collection period was conducted at 12th week of the study period to assess the digestibility coefficient of nutrients. Representative samples of kid starter and green grass offered were taken daily during the digestion trial for chemical analysis. The balance of feed and grass samples were collected from individual animals and their moisture content was determined daily. At the end of the collection period feed samples collected daily were pooled and subjected to chemical analysis. The dung was collected manually as and when it was voided. All precautions were taken to collect the dung quantitatively, uncontaminated with urine, feed residue or dirt. The dung collected each day was weighed accurately and were kept in double lined air tight plastic bags and stored fresh in deep freezer during the entire collection period. At the end of collection period daily samples stored from each animal were pooled and used for chemical analysis. Kid starter, fodder and dung samples were analyzed for proximate principles (AOAC, 2012). The acid detergent fiber (ADF) was estimated by the method suggested by Van Soest (1963) and neutral detergent fiber (NDF) by the method suggested by Van Soest and Whine (1967).

Blood samples were collected from all animals at the end of the experiment. These samples were used to determine haemoglobin, plasma total protein (Jong and Vegeter, 1950), calcium, phosphorus (Bernhart and Wreath, 1955), plasma cholesterol(Lie *et al.,* 1976) and triglycerides (McGowan *et al.,*1983) Blood haemoglobin was estimated by cyanomethaemoglobin method using reagents from Agappe diagnostics Ltd, Ernakulam, India. Plasma protein, calcium, phosphorus, serum cholesterol and serum triglycerides were determined using the blood analyzer (Mispa plus, SEAC radim group) and kits supplied by same suppliers. Data gathered on various parameters were analyzed statistically using Analysis of Variance (Snedecor and Cochran, 1994).

Results and Discussion

From the data collected on daily dry matter intake, the weekly average daily dry matter intake of the kids were 0.24, 0.24 and 0.22 kg , respectively in T_1, T_2 and T_3 initially and the corresponding values at the end of feeding trial were 0.56, 0.48 and 0.52 kg, which were similar.

Table 1. Proximate composition of Ksheerabala residue (%on dry matter basis)

Dry matter	92.55
Crude protein	29.52
Ether extract	13.26
Crude fibre	6.39
Total ash	8.42
Nitrogen Free Extract	42.41
Acid insoluble ash	0.06

Nutrient digestibility

Digestibility of dry matter, crude protein crude fibre, ether extract, nitrogen free extract, NDF and ADF observed in the present study were 77.34, 76.43 and 75.28, 81.97, 81.36 and 79.66, 64.47, 64.29 and 61.61, 81.33, 82.78 and 84.65, 84.04, 82.58 and 81.65, 59.77, 58.20 and 57.86, 55.58, 55.23 and 52.78 per cent in T_1, T_2 and T_3 kids, respectively and statistical analysis did not reveal any significant difference between the groups (Table 3). In agreement with the present result, Rani *et al.* (2006) reported that digestibility of DM, CP, NDF and ADF were similar in buffalo calves supplemented with two herbs namely bringraj (*Eclipta alba*) and kutki (*Kutaki picorrhiza*) at the rate of 0.4 per cent of dry matter intake. Verma *et al.* (1995) who also found that inclusion of water washed neem seed kernel cake at 0, 15 and 25 per cent levels in goat ration had similar effect on digestibility of DM, CP, EE and NFE.

Obeidat and Gharaybeh (2011) observed that the digestibility coefficient of DM and CP were similar in kids fed with diet containing 0, 10 and 20 per cent of sesame hull whereas the EE digestibility was significantly higher in treatment groups compared to control. Tufarelli *et al.* (2012) also reported that digestibility of CP and EE were similar in lambs fed with partly destoned exhausted olive cake

Table 2. Ingrediet and chemical composition of kid starters

Ingredients	Percentage composition of calf starter		
	T_1	T_2	T_3
Maize	35	29	23
Wheat bran	25	26	28
Soya bean meal	28	23	17
Dried fish	9	9	9
Ksheerabala residue	0	10	20
Salt	1	1	1
Calcite	2	2	2
Total	100.00	100.00	100.00
Vitamin Supplement g/100 kg feed	25	25	25
Trace mineral mix g/100 kg feed	25	25	25
Toxin binder g/100 kg feed	100	100	100
Salinomycin sodium g/100 kg feed	50	50	50
Chemical composition*			
Dry matter	92.19	91.73	91.93
Crude protein	23.74	23.99	24.51
Ether extract	4.95	5.38	6.04
Crude fibre	5.43	5.71	6.08
Total ash	9.72	10.07	10.41
Nitrogen free extract	55.65	54.84	52.87
Acid insoluble ash	1.11	1.23	1.28
Neutral detergent fibre	22.34	22.83	25.84
Acid detergent fibre	6.61	7.81	9.48
Calcium	1.03	1.12	1.18
Phosphorus	0.65	0.70	0.86

*On dry matter basis

Table 3. Digestibility coefficient of nutrients in experimental ration*

Item	Dietary treatments		
	T_1	T_2	T_3
DM	77.34 ± 0.21	76.43 ± 1.11	75.28 ± 1.26
CP	81.97 ± 0.85	81.36 ± 1.57	79.66 ± 1.03
CF	64.47 ± 0.84	64.29 ± 2.55	61.61 ± 1.56
EE	81.33 ± 0.97	82.78 ± 1.23	84.65 ± 0.94
NFE	84.04 ± 0.26	82.58 ± 1.19	81.65 ± 1.83
NDF	59.77±0.61	58.20±3.01	57.86±1.57
ADF	55.58±1.05	55.23±2.67	52.78±1.64

*on dry matter basis

Table 4. Haematological parameters of experimental kids

Parameters	T_1	T_2	T_3
Haemoglobin, g/dl	11.88 ± 0.31	12.00 ± 0.46	11.94 ± 0.57
plasma protein, g/dl	6.38 ± 0.19	6.33 ± 0.2	6.28 ± 0.25
Calcium, mg/dl	10.30 ± 0.11	10.31 ± 0.15	10.24 ± 0.32
Phosphorus, mg/dl	6.02 ± 0.12	6.02 ± 0.13	5.96 ± 0.29
Cholesterol, mg/dl	110.83 ± 2.96	104.03 ± 2.93	108.62 ± 6.23
Triglyceride, mg/dl	40.69 ± 0.41	39.36 ± 0.9	40.53 ± 1.3

at 0, 10 and 15 per cent levels in ration whereas the digestibility of DM was significantly lower in both diets containing olive cake compared to control.

Haematological parameters

Data on haematological studies were documented in Table 4. The average blood haemoglobin and plasma protein concentration at the end of the experiment for group T_1, T_2 and T_3 were 11.88, 12.00 and 11.94, 6.38, 6.33 and 6.28g/ dl, respectively. The average serum calcium values in experimental animals were 10.30, 10.31 and10.24 mg/dl for group T1, T_2 and T_3, respectively. The average serum phosphorus concentrations in the experimental kids were 6.02, 6.02 and 5.96 mg/dl for group T_1, T_2 and T_3, respectively. The average serum cholesterol and triglyceride values recorded at the end of the experiment were 110.83, 104.03 and 108.62, 40.69, 39.36 and 40.53 mg/ dl, respectively for group T_1, T_2 and T_3. There was no significant difference (P >0.05) in any of the haematological parameters between the three groups.

Verma *et al*. (1995) studied the effect of dietary inclusion of water washed neem seed kernel cake in growing goat ration at 0, 15 and 25 per cent levels and found that haemoglobin and cholesterol concentration were similar in treatment and control groups which is in accordance with the present result but total protein concentration was significantly lower in goats fed with neem seed kernel cake. An *et al.* (2001) found that the total cholesterol level was significantly reduced (113.92 mg/ dl) in broilers fed with ration containing 10 per cent red pepper seed oil meal compared to control diet (137.5 mg/ dl). Jasmine *et al*. (2018) reported similar values for haemoglobin, total protein, serum calcium and phosphorus in growing kids fed with ration containing spent rosemary (*Rosmarinus officianalis*.*L*) leaves

Conclusion

Critical evaluation of the results obtained in the present study revealed that inclusion of Ksheerabala residue in kid starter had similar effect on nutrient digestibility and haematological parameters. On summarizing the overall results of the study, it could be inferred that Ksheerabala residue can be included in the goat ration up to 20 per cent level without any adverse effect on nutrient digestibility and blood biochemical profile.

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RESEARCH ARTICLE
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Exome wide variant discovery by next generation DNA sequencing in Vechur cattle of Kerala*

R. S. Reshma[1], T. V. Aravindakshan[2], G. Radhika[3], T. Naicy[4] and K. Raji[5]
School of Applied Animal Production and Biotechnology,
College of Veterinary and Animal Sciences, Mannuthy-680 651, Thrissur, Kerala.

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Abstract

Vechur, the native cattle breed of Kerala, India is well-known for economically valuable phenotypic traits like disease resistance, adaptability to hot and humid tropical climatic conditions, low feed requirements and high quality milk. However, genomic information underlying these traits is rare. In the present study, the whole exome sequencing of a Vechur cow using Illumina HiSeq 2500 platform is reported. Comparison of sequences with Bos taurus reference genome assembly (UMD 3.1) identified 1,716,847 variants including 1,578,749 Single Nucleotide Polymorphisms and 138,098 Insertion/Deletions, of which 359,034 variants (20.91%) were novel. Detailed annotation of the identified variants showed that majority were situated in the intergenic region. Out of the 724,808 variants found inside the gene region, 107,880 were exonic variants. In the exonic variant, substantial proportion of non-synonymous (34.83%), frameshift (12.47%), nonsense (0.3%), start loss (0.06%) and stop loss (0.0009%) variants were identified. This information will provide a better understanding of genetic differences responsible for the peculiar phenotypic traits inherent to Vechur cattle.

Keywords: *Whole-exome sequencing, Vechur cattle, disease resistance, adaptability*

The cattle breeds in the world occur as two major subspecies – *Bos taurus* and *Bos indicus* which diverged from each other, more than 10,000 years ago from a common ancestor Auroch (*Bos primigenius*) (McTavish *et al.,* 2013). When compared to *Bos taurus* (hump less – European, Asian and African), *Bos indicus* (humped – South Asian and East African) is highly adapted to survive well in tropical and sub-tropical environmental conditions. Of the 50 registered cattle breeds of India surviving in various geographical

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1. *MVSc scholar, Animal Biotechnology, CVAS, Mannuthy. Ph.7902875712*
2. *Director, Centre for Advanced studies in Animal Genetics and Breeding*
3. *Associate Professor, Centre for Advanced studies in Animal Genetics and Breeding*
4. *Assistant Professor, Department of Animal Genetics and Breeding and corresponding author Ph.No. 9446119307, naicy@kvasu.ac.in*
5. *Associate Professor, Department of Veterinary Physiology*

and agro climatic regions (http://www.nbagr.res.in/regcat.html), Vechur (*Bos indicus*) is the cattle breed indigenous to Kerala, the southernmost state of India. This small cattle breed is noted for certain physiological traits like high disease resistance, low feed requirements, heat tolerance and adaptability to hot climatic conditions (Raghunandanan, 2006). In order to reveal the essence of these features, the genome wide analysis of Vechur cattle is needed.

After the completion of cattle genome sequencing, a number of genome sequencing studies were performed in different cattle breeds – Black Angus and Holstein (Stothard *et al.,* 2011), Fleckvieh and Braunvieh (Schwarzenbacher *et al.,* 2016), Hanwoo, Yanbian (Choi *et al.,* 2014 and 2015) Jeju Heugu and Korean Holstein cattle (Choi *et al.,* 2014) resulting in the identification of large number of different genomic variants. Apart from these, there are still a number of cattle breeds whose genome has not been well scanned. Understanding these genetic variations will be an important initiative to reveal the genomic information underlying trait variations.

With the development of massively parallel sequencing, now it is not difficult to obtain the genomic information of ecologically important organisms. Recent advances in sequencing technology established a basis for the understanding of evolutionary biology, ancestry and economically important phenotypic traits in animals. Now the emergence of Next generation DNA sequencing (NGS) technology have reduced the cost and increased the speed of sequencing by several orders of magnitude (Lee *et al.,* 2013). Regardless of the declining cost of sequencing, it is still expensive to perform whole genome sequencing of an eukaryotic organism like cattle having around 3 Gb genome size. So the cost effective approach is to sequence only the meaningful regions of the genome. Exome comprises 1 to 2 per cent of a typical eukaryotic genome depending on species (Warr *et al.,* 2015), representing the major portion for searching the variants with immense effect on phenotypic traits. With the advent of methods for separating exome DNA (Parla *et al.,* 2011), it is now feasible to sequence the exome genome wide. With this background, the present study was undertaken to sequence the exome of Vechur cow.

Materials and Methods

Library preparation and sequencing

Genomic DNA used for sequencing was extracted from the whole blood of Vechur cow. Since DNA quality was extremely important for obtaining quality sequence data, careful quality checking was applied for extracted DNA and DNA with A260/280 ratio of >1.8 was used for library preparation. DNA was sheared and exonic regions were captured using Agilent Sure Select- Bovine (All Exome - 54 Mb) oligonucleotide probes. It targeted 54 mega bases of bovine genomic DNA of interest. Captured fragments were adaptor ligated and produced libraries. Illumina HiSeq 2500 was used to generate paired end 2×100 bp sequences resulting in the generation of 3.22 Gb of raw sequence data. Of the total reads obtained, 92.7per cent had a phred quality score of 30 or more. Generated sequence data was subjected to variant calling.

Before alignment, quality distribution, base distribution and GC distribution of forward and reverse reads in paired end sequencing was checked from the fastq files. Quality assessment involved removal of low quality reads, contamination control and trimming of adaptor sequences (Nayarisseri *et al.,* 2013). Based on quality report, sequence reads were trimmed to retain only high quality sequence. Reads were aligned to the reference *Bos taurus* genome and gene model downloaded from Ensembl (ftp://ftp.ensembl.org/pub/release-81/gtf/bos_taurus/Bos_taurus.UMD3.1.81.gtf.gz) after trimming the raw reads based on base quality, base composition and adapter sequences with Burrows – Wheeler Aligner (BWA) (O'Rawe *et al.,* 2013) Version - 0.7.5. Aligned reads were sorted with Picard tool Version – 1.100 Sort Sam command. Picard Mark Duplicates command was used to

remove the read duplicates. SNPs and short indels were identified using SAMtools (Li *et al.,* 2009) Version – 0.1.18. Identified variants were further subjected to detailed annotation.

Results and Discussion

Sequencing and Aligning

In the present study, only the coding region of Vechur genome was targeted for sequencing. On an average 3.22 Gb of raw sequence data was generated as paired end 100 bp read length. The raw data quality summary is provided in Table 1. Low quality and contaminating reads were common artifacts in raw sequence data which would adversely affect the downstream analysis (Zhou *et al.,* 2013). Hence, quality control (QC) was critical for this NGS raw data. Out of the total 64,507,890 reads obtained, 64,506,674 (99.99%) reads passed QC.

When mapped to the *Bos taurus* reference genome (UMD 3.1), 98.44 per cent (63,500,350 reads) of the reads from sample aligned accurately (Table 1). Presence of duplicates might produce bias in variant allele identification and should be removed before variant calling, thereby reducing false calls and improving variant detection accuracy (Gao *et al.,* 2015). Duplicate reads were identified and removed. Chromosome wise coverage and dept is given in Table 2. It has been estimated that a minimum of 20 – 30 fold coverage is essential for detection of almost all variants (Li *et al.,* 2008). The coverage obtained was sufficient for detecting the variants.

Single Nucleotide Polymorphisms (SNPs) detection

Stringent QC measures were applied for reducing false positive variant detection. Variant calling using SAM tools identified 1,578,749 SNPs (91.95%). SNP is the abundant variant, controlling variations in traits of interest in cattle (Choi *et al.,* 2013). Of the SNPs identified 70.36 per cent were homozygous and 29.63 per cent were heterozygous. Low proportion of heterozygous SNPs may be due to strict variant calling requirements (Eck *et al.,* 2009). The variants were compared with dbSNP

Table 2: Chromosome wise coverage and dept

Chromosome	Coverage	Depth
1	22.57	2.05
2	24.21	2.36
3	26.69	2.7
4	23.53	2.09
5	26.33	2.56
6	22.38	2.06
7	26.12	2.42
8	24.53	2.06
9	22.13	1.91
10	25.98	2.66
11	27.83	2.48
12	22.06	1.77
13	28.81	2.3
14	24.55	1.96
15	25.86	2.43
16	27.25	2.32
17	25.87	2.13
18	33.23	3.07
19	35.3	3.55
20	23.58	1.94
21	27.3	2.11
22	28.17	2.42
23	28.39	2.47
24	24.94	1.85
25	36.02	3.08
26	27.15	2.43
27	24.78	1.98
28	26.17	2.22
29	29.45	2.47
X	22.16	1.62

Table 1: Raw read and alignment summary

Sample	Read orientation	Raw reads (paired end)	Bases (Gb)	GC%	Total reads	QC passed reads	Aligned read count	Properly paired
Vechur	R1	32,253,945	3.22	43.72	64,507,890	64,506,674 (99.99%)	63,500,350 (98.44%)	96.56%
	R2	32,253,945	3.22	43.72				

Table 3: Statistics of dbSNP filtered variants

Sample	Vechur
With dbSNP	1,357,813 (79.09%)
Without dbSNP	359,034 (20.91%)
Total	1,716,847

Table 4: Variant calling summary

Sample Name	Vechur
Total variants	1,716,847
Total SNPs	1,578,749 (91.95%)
Total indels	138,098 (8.04%)
Total homozygous SNPs	1,110,818 (70.36%)
Total heterozygous SNPs	467,931 (29.63%)
Total transition type SNPs	1,141,587 (72.30%)
Total transversion type SNPs	437,162 (27.69%)
T_S/T_V	2.61

after downloading the dbSNP files from NCBI (ftp://ftp.ncbi.nih.gov./snp/organisms/cow_9913/VCF/). As expected there was apparent difference between the *Bos taurus* and Vechur coding sequences. The comparison identified 359,034 novel variants and is summarized in Table 3. SNPs will occur either as transitions (purine to purine or pyramidine to pyramidine) or transversions (purine to pyramidine or vice versa). The total number of transition changes (T_S) observed was more than twice as compared to transversion changes (T_V). The whole exome transition to transversion ratio was calculated to be 2.61. During SNP detection studies using massively parallel sequencing technology, limitations in sensitivity and specificity can be expected. T_s/T_v ratio is helpful in assessing the errors in sequencing (Kraus *et al.,* 2011). A higher value is indicative of higher quality SNP calls (Liu *et al.,* 2012). The obtained T_s/T_v was close to the expected ratio for exome sequencing. Variants identified were summarized and provided in Table 4.

Insertion/deletions (Indels) detection

In the present study we identified 138,098 Indels (8.04%), out of which majority were insertion or deletion of a single base pair.

Variants having a depth of ≥5 and Q score of ≥20 were high quality variants (Bodi *et al.,* 2013). From total 1,716,849 variants (1,578,749 SNPs and 138,098 indels) identified, 825,916 SNPs (52.31%) and 93,559 indels (67.75%) were having a Q score of >20. When depth of identified variants were analyzed 606,886 SNPs (38.44%) and 57,147 indels (41.38) were with a depth of >5.

Variant annotation

Out of the total SNPs (1,578,749) identified, 724,808 SNPs (45.91 %) were found inside the genes, of which 107,880 SNPs (14.88%) were in exonic region and 616,928 (85.12%) were in intronic region. Exonic SNPs consist of SNPs in the coding region, non-coding gene, non-coding RNA (ncRNA) and untranslated region (UTR). There were 91,442 coding region SNPs (84.76%), 2,399 non-coding gene SNPs (2.22%), 454 SNPs (0.42%) in ncRNA and 13,585 SNPs (12.59%) in UTR. Out of 13,584 UTR SNPs, 3,053 (22.47%) were in 5'UTR and 10,532 (77.53%) were in 3'UTR. In 5-splice site 289 SNPs and in 3-splice site 417 SNPs were identified.

Out of the total indels (138,098) identified, 66,005 indels (47.8%) were identified to be inside the genes, of which 3,620 (5.48%) were in exonic region and 62,385 (94.51%) were in intronic region. Indels in the exonic regions were composed of coding region indels, non-coding gene indels, indels in ncRNA and UTR indels, including 1,852 coding region indels (51.16%), 159 non-coding gene indels (4.39%), 41 indels (1.13%) in ncRNA and 1,568 indels (43.31%) in UTR. UTR indels (1,568 indels) consisted of 319 (20.34%) 5'UTR and 1,249 (79.66%) 3'UTR indels. There were 137 indels identified in 5-splice site and 158 indels in 3-splice site.

Functional annotation of variants

The variant functional annotation identified 62,693 synonymous mutation, which may not significantly affect the phenotype of

an organism, but can affect protein folding and function (Parmley and Hurst, 2007). Also 37,564 missense mutations were detected that leads to non-synonymous amino acid substitutions. There were 1,345 frame-shift mutations leading to addition or deletion of a base pair or base pairs resulting in an alteration of reading frame from the position of mutation. Nonsense mutation which cause change of a sense codon to a chain-terminating codon was identified to be 322 numbers. Annotation also detected 67 start loss and a single stop loss variant.

Conclusion

Vechur cattle is renowned for its immunity, adaptability to local environmental conditions and high quality milk. During late twentieth century, cross breeding program of indigenous cattle breeds of Kerala with exotic high producing breeds with the objective to increase productivity, drastically reduced the population size of Vechur cattle. Now it is being realized that these cross bred cattle although have high productivity, lacks important traits required for better adaptability to hot humid conditions of Kerala. There is a growing demand for conserving this declining local genetic resource. Researches on complex traits have been benefited with the rising of NGS technologies. To reveal the genetic basis of phenotypes inherent to Vechur cattle, exome of Vechur cow was sequenced and mapped to *Bos taurus* reference genome assembly which generated more than one million variants. Exons although consists of only very small fraction of the complete genome, it had got a tremendous impact on the phenotype of an individual. Detailed analysis of this result identified 1,346 frameshift, 37,564 nonsynonymous, 323 nonsense, 66 startloss and a single stoploss variations which will lead to amino acid changes in the final protein product and can result in significant impacts on phenotype. From the total variants, 359,034 were novel including a number of nonsynonymous, frameshift and nonsense variants which might be responsible for the peculiar and unique phenotypic traits of Vechur cattle.

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RESEARCH ARTICLE
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Comparative analysis of milk quantity and quality in native goat breeds of Kerala*

K. Mathivathani[1], G. Radhika[2], T.V. Aravindakshan[3], R. Geetha[4] and Marykutty Thomas[5]
Department of Animal Breeding Genetics and Biostatistics,
College of Veterinary and Animal Sciences,
Mannuthy, Thrissur, 680 651

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Abstract

Goat rearing occupies an important place in augmenting the economy of our country. In India there are 23 well defined goat breeds and Kerala has two native breeds namely Malabari and Attappady Black, which vary significantly in milk production. Data on quantity of milk was collected from 100 Malabari and 70 Attappady Black goats. There was a significant influence of breed (p≤0.01) and parity (p≤0.05) on milk production. The average milk yield of Malabari goats was, 558.94 ± 4.21 *ml per day, while for Attappady Black it was* 340.22 ± 4.45 *ml per day. Milk samples were collected from 54 Malabari and 35 Attappady black goats and significant difference was observed in fat per cent between two groups. Double digest restriction associated DNA sequencing (ddRADseq) is a modern reduced representation sequencing technique which is a powerful and inexpensive approach for identification of Single Nucleotide Polymorphisms (SNP) across populations. This technique was applied on two groups of Malabari and Attappady Black goats with differing milk production and the preliminary evaluation of results revealed SNPs in five major genes affecting milk production.*

Keywords: *Milk production, quantity of milk, Malabari, Attappady Black, fat per cent, ddRADseq, SNP*

Goats are recognised as important components of livestock farming system and it plays a very useful role in giving a steady income to people of economically backward sections throughout a year. India has a goat population of 128 million, which is about 20 per cent of the total global goat population. India is having 23 well recognized goat breeds and Kerala has two native goat breeds namely, Malabari and Attappady Black. Malabari goats have medium sized body with high milk

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1. *MVSc scholar and corresponding author (Email:mathikvathani.mvc@gmail.com, Ph:7708788661)*
2. *Assistant Professor*
3. *Professor and Head*
4. *Assistant professor,Department of Dairy Science*
5. *Assistant Professor*

production and are reported well adapted to any climatic condition (Verma *et al*., 2009). Malabari goat breed is as the most popular dual purpose breed of Kerala (Bindhu and Ragavan, 2010). Attappady Black goats were reared by tribal community of hilly region of Attappady, Kerala. This breed is predominantly black in colour. It had long drooping ears with medium sized body (Stephen *et al*., 2005). Attappady black goats had less than 200 mL milk production per day (Mathew *et al*., 2005).

In India, annual milk production from goats was reported to be 15.2 million metric tonnes during 2014-15 as per Basic Animal Husbandry and Fisheries Statistics (2015). Goat milk is recommended for infants and convalescent people (Haenlein, 1992). This milk was an alternative source of animal protein for people with cow milk intolerance, and average diameter of individual fat globules in goat milk (2.76) was smaller than of cow milk (3.51) (Tziboula - Clarke, 2003), thus increasing its digestibility.

In the present study, milk quantity and quality were compared between Malabari and Attappady Black breed. Milk quality was compared between breeds during different lactation stages, namely early, middle and late. Double digest Restriction Associated DNA sequencing (ddRADseq) is modern reduced representation sequencing method for identification of Single Nucleotide Polymorphims (SNP) with low cost and high efficiency (Peterson *et al*., 2012). This technique was applied on Malabari and Attappady Black goats and on preliminary analysis of results five SNPs were identified in five major candidate genes affecting milk quantity and quality traits in goats

Materials and Methods

For milk quantity analysis, milk production records from the date of kidding to the date of drying were collected from 100 Malabari and 70 Attappady Black goats from University Goat and Sheep farm, Mannuthy. Animals were in first or second parity maintained at University Goat and Sheep farm, Mannuthy. Milk production per day was calculated for each goat by dividing the total milk production by number of days in milk yield. Milk Samples were collected from 54 Malabari and 35 Attappady Black goats from University Goat and Sheep farm, Mannuthy randomly. Milk samples were collected during early (28-30thday), middle (58-60thday) and late lactation (88-90th day) stages. The milk samples were cooled to refrigeration temperature. After thorough mixing all milk samples were analysed for fat, protein and lactose content immediately using milk analyser (mro scientific instrument) with frequent standardization using Gerber's method (BIS, 1981) for fat, Kjeldahl method (AOAC, 2012) for protein and Lane-Eynon method (BIS, 1981) for lactose.

In Double digest Restriction Associated DNA sequencing, whole genomic DNA digested with two types of restriction enzymes- rare cutter and common cutter. Ligation was done using adaptors, size selection of the products and PCR amplification was performed followed by Illumina sequencing, and further SNP analysis using bioinformatics tools. This technique was applied on two groups of Malabari and Attappady Black goats with differing milk production and the preliminary evaluation results revealed SNPs in five major candidate genes affecting milk production.

Statistical Analysis

Effect of breed and parity on average milk yield was analysed using fixed General Linear Model (GLM) of SPSS V.24. Fixed model used for milk production is given below

$Y_{ijkl} = \mu + b_i + p_j + h_k + e_{ijkl}$

Where,

Y_{ijkl} is the milk production measured on $ijkl^{th}$ animal

μ is the population mean

b_i is the fixed effect associated with i^{th} breed (i = 1,2)

p_j is the fixed effect associated with j^{th} breed - parity (j = 1 or 2)

h_k is the fixed effect associated with k^{th} parity-breed (k = 1 or 2)

e_{ijkl} is the random error

Milk quality was analysed using fixed GLM of SPSS V.24.

$$Y_{ij} = \mu + b_i + e_{ij}$$

Where,

Y_{ij} is the milk production measured on ij^{th} animal

μ is the population mean

b_i is the fixed effect associated with i^{th} breed (i = 1, 2)

e_{ij} is the random error

Results and Discussion

Milk quantity analysis

Comparison of milk yield between Malabari and Attappady Black goats revealed that average milk yield of 100 Malabari goats in this study was, 558.94 ± 4.21 mL per day, while for 70 Attappady Black it was 340.22 ± 4.45 mL per day. The least square means with standard error for breed and parity are presented in the Table 1. The results showed statistically significant difference between these breeds in milk production (p≤0.01). Milk production is influenced by genetic and environmental reasons. In the current study, Attappady black goat is having lower milk yield and the reason might be genetic makeup of the animal. Peacock *et al*. (1999) reported that there are various factors affecting milk production including age, breed, body size, udder shape and size, litter size, nutrition, season of kidding, temperature and disease condition. According to their study, non- dairy goat breeds in the tropics had daily milk yield up to 0.5 L, whereas dairy breed of goats, such as Saanen, Alpine, Nubian and Toggenburg produced two-four litres per day. The result obtained in the present study was in accordance with the findings of Verma *et al*. (2009) who stated that the milk yield varied from 0.5 to 1.5 L/day in Malabari goats.

Table 1. Least square means of daily milk yield with standard errors

Factor	Daily Average milk yield (mL)
Breed	**
Malabari (100)	$558.94^{b} \pm 4.21$
Attappady Black (70)	$340.22^{a} \pm 4.45$
p-value	≤0.01

Figures in parenthesis shows number of observations

Means with different superscripts in same column differ significantly (**p-value≤0.01)

In dual purpose goat breed Malabari, breed - parity interaction had significant influence on milk yield (p≤0.05). The milk yield in first parity was 540.740 ± 4.03 mL per day in Malabari and it gradually increased in second parity to 577.130 ± 7.37 mL per day. Crepaldi *et al*. (1999) reported the effect of parity on milk yield and stated that milk yield gradually increased from first to fourth lactation. Similar findings were reported by Idowa and Adewani (2017). The above results in the present study with regard to effect of parity on milk yield were in accordance with findings of Crepaldi *et al*. (1999) and Idowa and Adewani (2017). But in Attappady Black goats, breed – parity interaction had no significant influence on milk yield. The milk yield in first parity was 342.97 ± 5.21 mL per day in Attappady Black, and it decreased in second parity to 337.45 ± 7.22 mL per day. This result disagreed with the results of Crepaldi *et al*. (1999) and Idowa and Adewani (2017). Since Attappady Black is a meat type breed with low milk production, it milk yield did not increase with progress in parity, which might be the reason for no significance breed – parity interaction. In both breeds, parity – breed interaction had significant influence on milk yield ((p≤0.05). The milk yield in first parity was 540.740 ± 4.03 mL per day in Malabari, and 342.978 ± 5.21 mL per day in Attappady Black, which was significantly different. The milk yield in second parity was 577.130 ± 7.37 mL per day in Malabari, and 337.458 ± 7.22 mL per day in Attappady Black, which also shows significantly different. This clearly indicated that Malabari goats had high milk production than Attappady Black goats, in first and second parities, and hence parity – breed interaction had significant effect.

Breed	Parity 1	Parity 2
Malabari (100)	$540.740^{aA} \pm 4.033$	$577.130^{bA} \pm 7.379$
Attappady (70)	$342.978^{aB} \pm 5.218$	$337.458^{aB} \pm 7.224$

Means with different superscript (a - b in rows, A – B in columns) differ significantly in 5% level.

Milk quality analysis

Milk samples were collected from 54 Malabari and 35 Attappady black goats in early (28-30th day), middle (58-60th day) and late (88- 90th day) lactation stages. Significant difference were observed in fat per cent (middle and late stages of lactation), lactose per cent (early, middle and late stages of lactation) and protein per cent (late stage of lactation) between Malabari and Attappady Black goats and the results are given in Tables 2, 3 and 4 respectively. The means of fat, protein and lactose contents in milk of Malabari, Attappady Black goats were within the range of recorded value for dairy goats. Both breeds showed significant variation in fat per cent in early, middle and late lactation. Consistent increase in fat content was observed in Malabari and Attappady Black goats, towards late lactation. Usually a general inverse relationship existed between milk yield and fat test.

Fat

The Malabari goats had 2.07 ± 0.10, 3.82 ± 0.09 and 4.16 ± 0.17 per cent of milk fat content in early (28-30th), middle (58-60th) and late (88-90th) stages of lactation, respectively and the Attappady Black goats had 2.17 ± 0.10, 4.17 ± 0.09 and 4.54 ± 0.09 per cent of fat content. In the present study, the milk fat content differed ($p \leq 0.05$) significantly between the breeds, during different stages of lactation. Anfantakis and Kandarakis (1980) reported higher fat values during early and late lactation, but lower in the middle stage of lactation. Milk fat per cent during early stage of lactation showed no significant difference between breeds, the reason might be comparatively more milk production by Attappady Black goats during early stage when compared to middle and late stages of lactation. Fat per cent showed significant ($p \leq 0.05$) breed differences during middle and late lactation. Current study revealed that Attappady Black breed of goats having lower milk yield, produced higher levels of fat content when compared with Malabari goats and the reason may be attributed to negative genetic correlation between milk yield and milk fat content.

Protein

In the current study, protein per cent in milk varied from 2.52 ± 0.06 to 3.08 ± 0.09 per cent in Malabari and 2.65 ± 0.06 to 3.15 ± 0.09 per cent in Attappady black goats in different stages of lactation. Significant difference in protein content was observed between breeds in late lactation but no significant change was seen in early and middle lactation. Singh and Singh (1980) analysed protein per cent in goat milk and reported an average of 2.9 per cent in early lactation, 3.2 per cent mid lactation and 3.8 per cent in late lactation. According to Kandarakis *et al*. (2001) protein and fat content in goat milk are high during colostrum stage, in early lactation, lower thereafter and rise again markedly at the end of lactation, when production is low. Generally, average per cent of goat milk components was influenced by feed, season, stage of lactation and genetic variation. In the present study, values observed for protein were within the expected range. Since both types of breeds were grown under similar farm condition, genetic variation may be the reason for the observed change.

Lactose

Lactose content showed significant variation between breeds in different stages of lactation. However, the values were within range. Qureshi *et al*. (1981) observed that the protein and lactose per cent of Jamunapari goat milk was 3.8 and 3.9 respectively. Boros (1986) and Simos *et al*. (1991) reported that protein and lactose were fairly constant over the different stages of lactation. According to researchers, lactose content usually remained constant under normal farm conditions.

ddRADseq results

Pooled DNA samples of Malabari and Attappady black goats were outsourced for ddRAD sequencing. Whole genome was digested with restriction enzymes, and after size selection of products, Illumina sequencing was performed. On further analysis using bioinformatics tools, SNPs were identified by tool Venny 3 and preliminary evaluation of results revealed five SNPs in five major candidate genes affecting milk quality and quantity.

Table 2. Least square means of milk fat per cent with standard errors

Group	Fat % Mean ± SE		
	Early	Middle	Late
Malabari (N=54)	$2.07^{a} \pm 0.10$	$3.82^{a} \pm 0.09$	$4.16^{a} \pm 0.17$
Attappady Black (N=35)	$2.17^{b} \pm 0.10$	$4.17^{b} \pm 0.09$	$4.54^{b} \pm 0.09$
(p-value)	≥0.05	≤0.05	≤0.05
Breed	NS	*	*

Table 3. Least square means of lactose per cent with standard errors

Group	Lactose % Mean± SE		
	Early	Middle	Late
Malabari (N=54)	$4.41^{b} \pm 0.09$	$4.21^{a} \pm 0.05$	$4.94^{b} \pm 0.06$
Attappady (N=35)	$4.09^{a} \pm 0.09$	$4.35^{b} \pm 0.05$	$4.63^{a} \pm 0.06$
p-value	≤0.05	≤0.05	≤0.05
Breed	*	*	*

Table 4 Least square means of protein per cent with standard errors

Group	Protein % Mean ± SE		
	Early	Middle	Late
Malabari (N=54)	3.08 ± 0.09	2.56 ± 0.05	$2.52^{a} \pm 0.06$
Attappady Black (N=35)	3.15 ± 0.09	2.65 ± 0.06	$2.76^{b} \pm 0.07$
(p-value)	≥0.05	≥0.05	≤0.01
Breed	NS	NS	*

Figures in parenthesis shows number of observations
Means with different superscripts in same column differ significantly (* p-value<0.05), NS- Non-significant

Major candidate genes affecting milk production from ddradseq

In the initial phase of study, screening of five major candidate genes for SNPs from ddRADseq results was performed.

1. Oxidized Low Density Lipoprotein 1 gene (OLR1)

Downstream variant of this gene c.*4533T>C was observed by ddRAD seq. Oxidized Low Density Lipoprotein 1 played as important role in lipid metabolism. This gene was reported to be involved in fattyacid transport during lactation cycle (Khatib *et al.,* 2006). OLR1 was a major protein, which affected milk production traits in cattle (Komisarek *et al*., 2009). Micro RNA (miRNA) binds with 3'UTR of OLR1 gene and altered expression of gene. Reports indicated that this variation of 3'UTR region was associated with milk production traits in cattle (Wang *et al.,* 2013).

2. Acetyl- Coenzyme A Carboxylase α gene (ACACA)

Intronic variant c.154+8944C>T was discovered by ddRAD seq. Acetyl- Coenzyme A Carboxylase α, the important regulator enzyme for biosynthesis of fatty acids, catalysed the conversion of acetyl-CoA to malonyl-CoA. Gene *ACACA* was found to be markedly up regulated during lactation in cattle (Bionaz and Loor, 2008).

3. Lipoprotein lipase gene (LPL)

An intronic variant c.243+1082C>T was identified in sixth intron of *LPL*, by

ddRADseq. Lipoprotein lipase was an enzyme in milk which was responsible for enzymatic lipolysis, *i.e*. the hydrolysis of fatty acids from triglycerides and phospholipids in milk. (Barber *et al*., 1997).

4. GLI Family Zinc Finger 2 gene (GLI2)

Exonic variant c.64T>G of this gene was obtained by ddRADseq. Candidate gene *GLI2* was related with udder, and this gene was responsible for mammary gland development, mammary gland duct morphogenesis, mammary gland alveolus development and epithelial tube morphogenesis. (Marete *et al*., 2018).

5. Butyrophilin gene (BTN1A1)

Upstream gene variant c.-11528G>A of this gene was observed by ddRAD seq. Butyrophilin is a transmembrane glycoprotein, specifically expressed on the apical surface of the mammary epithelial cells during lactation. This protein was mainly associated with milk fat droplets and was assumed to be involved in secretion of fat globules, in mammary epithelial cells (Jack and Marther, 1990).

One SNP each was identified on five major candidate genes affecting milk production in goats, of which one was an exonic variant (*GLI2*), One downstream gene variant (*OLR1*), one upstream gene variant (*BTN1A1*) and two were intronic variants (*LPL, ACACA*).

Conclusion

Comparsion of milk production between Malabari and Attappady Black goats, revealed that average milk yield of Malabari goats was higher (558 mL per day) compared with Attappady Black goats (340 mL per day). Regarding milk quality analysis, milk fat content significantly differed between the breeds, during middle and late stages of lactation. ddRADseq technique was applied on two groups of Malabari and Attappady Black goats with differing milk production and the preliminary evaluation of results revealed SNPs in five major genes affecting milk production.

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SHORT COMMUNICATION
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Response of crossbred cattle in terms of respiration rate and rectal temperature to maximum and minimum THI period in Kerala*

N.V. Jisha[1], G. Girish Varma[2], V. Beena[3], A. Prasad[4], V.L. Gleeja[5], K. Karthiayini[6] and V. Sejian[7]
Centre for Animal Adaptation to Environment and Climate Change Studies (CAADECCS)
College of Veterinary and Animal Sciences, Mannuthy, Thrissur - 680 651,
Kerala Veterinary and Animal Sciences University

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The present study assesses the relationship of meteorological variables with physiological stress parameters. Respiration rate was continuously measured (RR) using Physiological monitor with improvised facemask. Rectal temperature (RT) was continuously measured using in- dwelling thermo couple probes attached with data acquisition system. The experiment was conducted in the Livestock Farm of Kerala Veterinary and Animal Sciences University, Mannuthy, Thrissur, Kerala. Experimental animals included eight, six months to one year old crossbred female cattle. The study was conducted for a period of 120 days in two seasons, season I and season II covering maximum Temperature Humidity Index (THI) and minimum THI seasons (Season I: 24th March 2017 to 25th May 2017 and Season II: 15th December 2017 to 13th February 2018).

Regarding the physiological response of animals to the prevailing 'seasons' RR is the first one to show changes and hence the first sign of strain. Respiratory rate started increasing at ambient temperature of 30^{0}C and THI of 82. Increase in respiratory rate was found to be the first level of defense to prevent the increase in body temperature as suggested by Kabuga (1992). The accelerated RR may be a thermoregulatory response to increase evaporative heat loss to maintain core temperature (Marai *et al*., 2007).

When the atmospheric temperature increases, panting starts in animals as the first physiological mean of thermoregulation. When the hot air traverse to the upper airways water molecules on surface of the air ways get evaporated taking the latent heat of evaporation from

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1. *Ph.D Scholar, CAADECCS, COVAS, Mannuthy.*
2. *Dean, College of Avian Science and Management, Thiruvazhamkunnu. girish@kvasu.ac.in, 9446229673.*
3. *Assistant Professor and Implementing officer, CAADECCS, COVAS, Mannuthy*
4. *Assistant Professor, Department of Livestock Production and Management, COVAS, Mannuthy*
5. *Assistant Professor, Department of Statistics, CVAS, Mannuthy.*
6. *Professor and Head, Department of Veterinary Physiology, CVAS, Pookode.*
7. *Senior Scientist, NIANP, Bangalore.*

Table 1. Correlation coefficients of physiological parameters with meteorological variables (Season I – Period of maximum THI and Season II - Period of minimum THI)

Parameters	Time	Season I		Season II	
		AH	RR	AH	RR
Temperature in (^{0}C)	Morning	-0.501*	0.458*	-0.195	0.131
	Evening	-0.418*	0.621**	-0.436*	-0.130
Relative humidity in (%)	Morning	0.408*	-0.476*	0.116	-0.262
	Evening	0.487*	-0.470*	-0.012	-0.231
THI	Morning	-0.376	0.428*	-0.309	-0.303
	Evening	-0.300	0.618**	-0.348	-0.393

**Correlation is significant at the 0.01 level. *AH – Absolute Humidity

* Correlation is significant at the 0.05 level * RR – Respiration Rate

Table 2. Comparison of Physiological parameters in season I and season II (Season I – Period of maximum THI and Season II - Period of minimum THI)

Parameters	Mean ± SE		t-value			t-value
	Morning			Evening		
	Season I	Season II		Season I	Season II	
Ambient Temperature	32.09 ± 0.46	27.97 ± 0.43	6.481**	33.47 ± 0.44	33.11 ± 0.20	0.752ns
Ambient Relative humidity in %	70.72 ± 1.85	66.57 ± 2.22	1.441ns	68.26 ± 1.64	47.26 ± 2.09	7.814**
THI	84.2 ± 0.53	77.48 ± 0.51	9.169**	86.20 ± 0.54	81.77 ± 0.34	7.058**
Absolute humidity in the expired air of the animal	90.32 ± 1.05	87.61 ± 0.77	2.089*	89.31 ± 1.11	75.56 ± 2.23	5.510**
Respiratory rate of the animal	53.97 ± 1.58	33.28 ± 0.91	11.337**	60.90 ± 2.49	37.24 ± 0.75	9.112**

*Means are significantly different at 5% level

** Means are significantly different at 1% level

ns-non significant

Table 3. Correlation coefficients of rectal temperature with meteorological variables (Season I – Period of maximum THI and Season II - Period of minimum THI)

Parameter	Time	In house temperature	In house humidity	Average of THI
Rectal temperature	Season I	0.351	-0.087	0.448*
	Season II	-0.204	-0.248	0.006

*Correlation is significant at 0.05 level. *THI – Temperature Humidity Index

Table 4. Comparison of Rectal Temperature (RT) of season I and season II (Season I – Period of maximum THI and Season II - Period of minimum THI)

Parameters	Season I	Season II	t-value
Rectal temperature	39.72 ± 0.085	39.19 ± 0.049	5.399**

**Means are significantly different at 0.01 level

In season I, RT was significantly different and higher than that of season II (Table 4).

the body. It induces a cooling effect on blood (Sherwood *et al*., 2012). When the fully saturated air comes out during expiration some part of the water vapour of the exhaled air may get condensed on the relatively cooler mucus membrane. When the atmospheric temperature increases the effect of evaporative cooling also increases indirectly increasing the water vapour condensation of the exhaled air on the mucus membrane of the upper air ways there by reducing the absolute humidity of the expired air. This may be the reason for the negative correlation between ambient temperature and absolute humidity of the expired air. But when the relative humidity of the atmosphere increases evaporative cooling of upper air ways become ineffective due to reduced gradient of water vapour partial pressure. This may lead to increase in the amount of water vapour of the expired air and forms the reason for the positive correlation between relative humidity of atmosphere and absolute humidity of expired air. So from these observations it can be inferred that increased respiration rate in conditions of increased THI due to elevated RH not only becomes the less effective thermoregulation in animals but also becomes an extra burden on the system due to increased energy consumption.

In season I and season II the ambient temperature are more or less the same and both exhibited negative correlation with absolute humidity of the expired air. In season I and season II, though the evening ambient temperature was more or less the same, increased relative humidity in season I showed a negative correlation with absolute humidity of the expired air. But in season II no correlation could be observed between RH of the atmosphere and AH of the expired air. So relative humidity affect evaporative cooling negatively, when it cross a particular limit. This limit could be identified by doing experiments at different RH levels using a climate chamber. The absence of correlation between THI and AH may be due to the contradictory correlation exhibited by the components on THI on absolute humidity

Summary

The observation indicated that from THI 82 to 86 the body of the animal could maintain body temperature by physiological means of thermoregulation primarily by increasing respiration. Rectal temperature showed a positive correlation when THI reaches 86 and rectal temperature started increasing at ambient temperature of 34^{0}C and THI 86. But beyond 86 all these mechanisms failed and body temperature started rising. The relative humidity affects evaporative cooling negatively when it cross limit. This limit is very important to adopt management practices and could be identified by doing experiments at different RH levels using a climate chamber. The absence of correlation between THI and AH is also pointing to the fact that temperature and humidity affect the evaporative mechanism independently.

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