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Observation of Biochemical Variations in Sheep (*Ovis aries*) Feaces during Different Reproductive Phases

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Abstract

Biochemical profile is a set of diagnostic procedures that are based on determining the various reproductive indicators of the animals. Female reproductive physiology is a complex process and the macromolecules produce from females depend upon hormonal regulation and physiological status of the organisms. The measurement of reproductive steroids and their metabolites excreted through urine and feaces serves as purpose of communication and provides new techniques for detection of effective estrus phase in animals. For a better understanding of physiological and biochemical processes correlated to chemical communication in mammals it is necessary to monitor the chemical characterization of pheromones together with biological testing frequently. Hence, the present investigation is designed to analyze the biochemical profiles such as protein, carbohydrate, lipid and steroid hormones such as estrogen, progesterone, LH, FSH in prepubertal, estrus, pregnant and lactating sheep feaces. The level of protein and lipid were significantly high in estrus phase, where as the level of carbohydrate level is significantly high in prepubertal and lactating phases when compared with other phases. Further, estrogen and LH were significantly high in estrus as compared with other phases and progesterone level is significantly high in pregnant phase. Thus, the present work is concluded that biochemical and hormones in estrus phase may represent as important elicit signals to inform the estrus phase of the animals.

Keywords: Pheromones, chemical communication, hormonal regulation, estrus phase.

Introduction

Countries like India, which has abundantly international livestock gene pool and has development in animal production. Domestication of animals was an essential step in human demographical and cultural development. Sheep which has a vital economic in India are mostly raised under harsh environment condition as it is seasonal breeders Bhatia and Arora[1]. Tamilnadu is home of eight recognized sheep breeds of which the Kilakarasal, Ramnad White and Vembu are distributed in southern agro climatic zone of Tamilnadu[2,3].

Chemical communication among individuals of the same species serves many important functions like sexual attraction Achiraman[4], Achiraman and Archunan[5,6,] Brennan and Keverne[7] interference with puberty, the estrous cycle, and pregnancy Dominic[8], Drickamer[9]. The odors produced from females may vary according to the reproductive phases Michael[10], O'Connell[11]. All mammals excrete chemical signals to the external environment through urine, saliva, faeces, and specialized scent glands Vandenberg[12]. The male has more attraction to estrus female than non- estrus due to the presence of some specific compounds Archunan[13]. The biochemical constituents along with pheromones may contribute along to variation across reproductive cycles and facilitates to estrus detection. The composition of the excretory products may vary according to the various reproductive phases. It is therefore believe that the biochemical constituent of excretory product vary during estrous cycle. Further, the role of hormones in regulation of biochemical constituent in excretory are not known Dominic[8].

The evaluation of urinary steroids is necessary to know the physiological condition of the animals Lokutoff *etal*[14], Steven *etal* [15]. There is limit in handling animals for investigation mainly for heifers and dry-off animals. Estimation of faecal steroid is one of the non- invasive methods to monitoring of reproductive status. Therefore, in the present study, the faecal steroids were estimated Schwarzenberger *etal* [16]. Primarily , for the study on metabolism and excretion the faecal steroid hormones were mainly analysed Adlercreutz *etal* [17], advanced studies done for monitoring management of zoo, domestic animals and in different reproductive stages of the animals Schwarzenberger *etal*[18].

The different phases of reproductive cycle are regulated by intricate sequential events and interactions between hypothalamic releasing hormones, they secreted from pituitary and sex steroids secreted by ovary. A sound knowledge of reproductive functioning in terms of interplay of hypothalamic, gonadotropic and gonadal hormones, with synergistic and antagonistic influences from other hormones and factors involved in the regulation of various reproductive stages, accurate estrus detection, timely pregnancy diagnosis and early detection of non conceived stock can be expected to an improvement of the reproductive efficiency Mondal *etal*[19].

In the reproductive stages of female like estrus, ovulation, pregnancy and parturition, the two important female sex steroid hormones estrogen and progesterone determines behaviour changes in the animal. The pattern of secretion of these hormones has been well-documented in cattle, buffalo, goat, mare and pig but is less well understood in sheep Sumar *etal* [20].

Determination of status and characterization of indigenous sheep essential domesticate animals for their conservation plans. The artificial insemination may be an unconventional method to ensure the fertilization process sucessfully, to overcome this, the information regarding female reproductive condition and the time of ovulation is necessary. The identification of biochemical constituents of faeces during various reproductive phases will evaluate the functional aspect of biochemicals. Hence the present work is to determine the level of biochemicals like protein, carbohydrate and lipid along with hormones in various reproductive stages of sheep.

Material and Methods

Animals: Six healthy sheep of Ramand White breed were used in the present study for sample collection in District Livestock farm, Pudukkottai, Tamilnadu, India, and fed with conventional diet and water *ad libidum.*

Estrus determination: The expression of estrus behaviour in the sheep is not easy to detect when compare with other cattle. The physical characteristics such as restless, reddened and swollen vulva, but this is often difficult to detect the estrus, because of the wool and small size of the vulva. Some of the secondary behaviours such as rapid tail movement and raised tailed in the presence of male is also considered as signs for estrus determination.

Sample collection: The feacal samples were collected from sheep during various reproductive phases at 8.00 a.m. The stages of the estrous cycle were carefully examined for two to three consecutive cycles. The sheep were considered to be in estrus if they accepted the mounting by another sheep. The samples were pooled and stored at -20^0 C for further studies.

Biochemical assay: The biochemical constituents such as protein Lowry *etal* [21], carbohydrate Dubois *etal* [22], lipid Folch *etal* [23], hormones such as estrogen, progesterone, LH and FSH by RIA Lafrance and Goff [24] during various phases like prepubertal, estrus, pregnant and lactation in sheep faeces were analysed .

Statistical analysis: The results of the biochemicals and hormones in different stages are presented as mean value ± SE and were subjected to statistical analysis with one way ANOVA test, Zar[25] followed by pos hoc Duncan's test Ducan[26]. All statistical analyses were performed using the statistical package for social science (SPSS) program at $p < 0.05$ level was considered as significance.

Results and Discussion

In analysis of biochemicals like protein, carbohydrate and lipid in sheep feaces, the following results were obtained. The level of protein is significantly high in estrus stage followed by lactation, prepubertal, and pregnant animals and this result is similar with the result of Achiraman and Archunan[27] urinary protein of mice, which is significantly differed across the different reproductive phases of female shows that estrus urine contained the highest level of protein followed by proestrus.

The level of carbohydrate is found to be significantly high in lactation and prepubertal animal feaces when compared to estrus and pregnant. The present result is supported with the earlier results of Jacob and Vadodaria[28], they stated that glucose concentration as in lactation ewe has to be considered as a result of constant energy loss with the milk. Low glucose level in pregnant is associated with foetus development and mobilization of maternal glucose to foetal blood circulation. These changes suggest that the combination of increased utilization of glucose for milk lactose synthesis and low intake of nutrient Pambu-Gollah[29].

In this investigation, the increased level of lipid was observed in estrus animal feaces when compared to all other phases. Similar results was also obtained by Achiraman and Archunan[27], in female mouse urine and bovine urine Rameshkumar and Archunan[30], Prabu and Rameshkumar[31] sheep urine Rameshkumar *etal*[32], and they found that higher amount of lipid was present in estrus urine followed by prepubertal and lactation. They also stated that the endocrinological status may act major impact in excretion of lipid and greater expression in estrus phase.

It is known that hormones have a rhythmic variation among the various reproductive phases, in the present work the significant level of faecal estrogen and LH are present in estrus phase, which is similar to the report of Wallen[33,] reported that high level of faecal estrogen is present in ovulatory phase. Urinary estrogen analyses are capable of characterize the two follicular waves that occur during the estrous cycle and also possible measurements of other steroid metabolites in serum, urine and faeces Czekala *etal*[34].

The level of progesterone is significantly high in pregnant stages when compared to prepubertal, estrus and lactation animals. The present result is correlated to the result of Ahmed *etal* [35], that progesterone levels rise and fall in coincidence with the growth and regression of corpus luteum (CL). Peripheral progesterone concentrations are minimal on the day of oestrus, rise to peak concentrations even on day 17 Ahmed *etal* [35]; Bachalaus *etal*[36] Pahwa and Pandey[37] before declining to basal levels at the onset of next oestrus. Hence, the present study concluded that the level of protein, lipid, estrogen and LH are higher in estrus stage when compared to all other stages may be a reason for estrus indicator.

Table-1
Biochemical constituents of sheep faeces during various reproductive phases

Biochemicals / Stages	Protein mg/g	Carbohydrate mg/g	Lipid mg/g
Prepubertal	0.22 ± 0.02 [a]	0.70 ± 0.01 [d]	0.07 ± 0.01 [a]
Estrus	0.70 ± 0.02 [c]	0.32 ± 0.02 [a]	0.47 ± 0.01 [b]
Pregnant	0.21 ± 0.01 [a]	0.35 ± 0.01 [b]	0.07 ± 0.01 [a]
Lactation	0.34 ± 0.01 [b]	0.63 ± 0.01 [c]	0.08 ± 0.01 [a]

Values are expressed in Mean ± SE, Dissimilar alphabets in vertical column are significantly different at P < 0.05% level.

Table-2
Hormonal profiles of sheep faeces during various reproduc tive phases

Hormones Stages	Estrogen ng/ml	Progesterone pg/ml	LH mlU/ml	FSH mlU/ml
Prepubertal	29.5 ± 0.02 [b]	7.2 ±0.03 [b]	0.26 ±0.02 [b]	0.92 ±0.02 [b]
Estrus	62.4 ± 0.01 [d]	0.41 ±0.01 [a]	5.32 ±0.02 [c]	3.12 ± 0.02 [c]
Pregnant	15.2 ±0.01 [a]	49.0 ±0.02 [d]	0.13 ± 0.01 [b]	0.81 ± 0.01 [b]
Lactation	48.0 ±0.01 [c]	10.5 ±0.01 [c]	0.06 ± 0.01 [a]	0.03 ± 0.01 [a]

Values are expressed in Mean ± SE, Dissimilar alphabets in vertical column are significantly different at P < 0.05% level

Conclusion

Thus, the present findings suggested the evaluation of endocrine status by faecal steroids is one of the important tools for efficient management, and efforts to use assisted reproductive technologies like artificial insemination, embryo transfer; diagnosis of reproductive disorders depend on the knowledge of basic reproductive physiology of particular species. To examine the reproductive status of the animals, faecal steroid investigation will helpful as a non-invasive tool. Based upon the evaluation of biochemical parameter it is possible to detect the metabolism and accurate detection of estrus phase in farm animals.

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