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Effect of ketosis on the thyroxine hormone levels in lactating buffaloes

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Abstract
The present study was an effort to find out the effect of ketosis on the serum Thyroxine (T_4) levels in buffaloes. The study was carried out in 18 ketotic buffaloes from dairy farms of Kuchaman city, Nagaur (India), 10 healthy buffaloes acted as control group. Statistical analysis revealed non-significant decrease in serum T_4 levels in ketotic buffaloes compared to healthy ones.

Keywords: Ketosis, buffaloes, thyroxine profile, metabolic disease, dairy animal

Introduction
Agriculture sector still acts as a main driver of rural economy and livestock contributes a large portion of draft power for agriculture, with approximately half the cattle population and 25% of the buffalo population being used for cultivation (19th livestock census). In Rajasthan, about 8% GDP is contributed by livestock sector alone (Dept. of Animal Husbandry, Rajasthan). This sector has greater potential for rural self-employment at the lowest possible investment per unit. However, diseases, specifically metabolic diseases impose a significant economic burden on the sector leading to significant losses in production and value erosion of livestock unit. Among key metabolic diseases, ketosis is a widely prevalent disease that affects cattle and buffaloes.

Ketosis is a multifactorial metabolic disorder of energy metabolism in high producing dairy animal during early lactation in both industrialized and developing countries. Ketosis is characterized by abnormally elevated levels of ketone bodies in body fluids (blood, urine, milk) and tissues [1], a reduction in blood glucose level and liver glycogen and an increased fat mobilization that results in elevated ketone body accumulation in blood [2]. Susceptibility to ketosis is probably due to the combination of appetite limitation and a high degree of precedence given to the demand of the mammary gland for nutrients, in particular glucose. Ketosis is caused by a negative energy balance and typically occur within two months after calving [1,3]. Feeding of commonly available poor-quality fodder coupled with high milk yield in buffaloes cause energy deficiency in animal and such energy deficiency occurring in periparturient period is suspected to induce ketosis in buffaloes.

Due to the losses associated with ketosis, it becomes imperative to devise strategies to prevent and diagnose the disease at the earliest. The diagnosis requires finding crucial markers that are implicated in the disease, the serum thyroxine is one such marker, that has been scrutinized for its use as a diagnostic marker for ketosis [4]. However, studies are still very limited and no clear conclusion is there on the thyroxine profile in relation to ketosis in animals. So, this study was designed to scrutinize the thyroxine profile in buffalo population of the semi-arid region in western Rajasthan.

Materials and Methods
In this study, the blood samples of ten healthy and eighteen clinically diagnosed ketotic adult buffaloes of Murrah breed were analysed. Recently parturiated buffaloes 4-8 years age, in their 3rd to 6th parity, weighing 400-480kg and clinically diagnosed with history of inappetence, pica and sudden fall in milk production were selected for this study. For confirmation of ketosis, the urine sample of suspected animal were analysed by means of Rothera's test and strip test. Ten clinically healthy buffaloes were also included from a dairy farm of Kuchaman city, Nagaur, which acted as control group.

Blood sample from ten healthy and eighteen Ketotic buffaloes were collected from jugular vein into plain tubes (not containing any anti-coagulants), the samples were left to clot at room

temperature, serum was separated by centrifugation and stored at -20 ^{0}C till further analysis. Thyroxine (T_4) in nmol/L was estimated by enzyme- immune assay (EIA) technique using pathozyme T_4 kit supplied by omega diagnostics, Scotland, UK, following manufacturer's protocol, the steps followed has been provided in following paragraphs.

Principle of assay

Specific anti Thyroxine (T_4) antibodies are coated onto microtitration wells and test sera are applied. Thyroxine (T_4) with Horseradish peroxidase enzyme (conjugate) is then added which competes with the released serum T_4 for available binding sites on the solid phase. After incubation, the wells are washed with water to remove any unbound T_4 or T_4 enzyme conjugate. On addition of the substrate (TMB), color develops only in those wells in which enzyme is present, indicating a lack of serum T_4. The reaction is stopped by the addition of dilute sulphuric acid and the absorbance is then measured at 450nm.

Assay Procedure

Prior to assay, all the kit components and plasma are brought to room temperature (20 ^{0}C to 35 ^{0}C). One set of standards should be run with each batch of test plasma. The desired number of coated wells were secured in the holder and position of standards and test plasma on the EIA microtiter plate was recorded in the sheet provided. 25µl of standards and test plasma were dispensed into the assigned wells, followed by addition of 100µl of working strength conjugate into each well. The components were thoroughly mixed for 30 seconds, followed by 60 minutes incubation at room temperature (20 ^{0}C to 25 ^{0}C). At the end of the incubation period, the contents of the wells were discarded by flicking plate contents into Biohazard container. The wells were filled with minimum of 300µl of wash buffer per well, emptied and struck sharply against absorbent paper, the step was repeated for 5 times for complete washing. For Machine washing, each well was again filled with 300µl of wash buffer and that an appropriate disinfectant was added to the waste collection bottle, the empty wells were washed 5 times. After washing, excess fluid was removed by striking the wells sharply on to absorbent paper or paper towel to remove all residual water droplets. 100µl substrate solution was then dispensed into each well and mixed gently for 5 seconds, followed by 20 minutes of incubation in the dark for at room temperature (20 ^{0}C to 25˚C). The reaction was stopped by adding 100µl stop solution to each well, gently mixed for 30 seconds to ensure that the blue colour changes completely to yellow colour. The optical density was read immediately (not later than 10 minutes) in a micro plate reader with 450 nm filters.

Calculation of T_4 Concentration

The mean absorbance value (A450) was calculated for each set of standards and specimens. A standard curve was constructed by plotting the mean absorbance for each standard against its concentration in ng/ml on graph paper, with absorbance values on the y-axis and concentration on the x-axis. The mean absorbance values were used for each specimen to determine the corresponding concentration of T_4 in ng/ml from the standard curve.

Statistical analysis

The data obtained were statistically analyzed and compared using standard formulae given for mean, standard error and Student t-test as per the procedures explained by Snedecor and Cochran (1968). Student t –test was used to compare the significances of the difference of each parameter between the control and ketotic groups.

Results and Discussion

The mean± SE concentration and t values of thyroxine (T_4) in healthy and ketotic buffaloes has been presented in table no. 1. The average serum thyroxine level was found to be 41.23 ± 3.05 and 38.48 ± 2.00 in control and ketotic buffaloes, respectively. On statistical analysis, the difference between thyroxine levels in serum of control and ketotic buffaloes was found to be non-significant, with ketotic buffaloes showing decreased serum thyroxine levels. The mean serum thyroxine (T_4) decreased by 6.67% in ketotic buffaloes in comparison to control buffaloes.

Table 1: Serum thyroxine levels in control and ketotic buffaloes

	Groups	Mean ±S.E.	%Inc./dec	t-Value
Serum Thyroxine (T_4)	Control (n=10)	41.23 ± 3.06	-6.67	0.283063
	Ketotic (n=18)	38.48 ± 2.01		

Dairy animals in early lactation are in a state of metabolic stress, in order to meet the needs of increased energy of mammary gland and to adjust the neuro-endocrine system to the new metabolic needs of the body. One of these endocrine factors is the thyroid hormone. The hormonal activity of thyroid gland has an important role in the transition period for determining the cell metabolism intensity, metabolism of lipids and carbohydrates and lactation course itself, regulated by thyroid hormones [5, 6]. Thyroxine has an important role on the carbohydrate metabolism due to increase glucose turn over and absorption. It plays upon a greater multiplicity of metabolic processes, influencing the concentration and activity of numerous enzymes; the metabolism of substrates, vitamins and minerals; the secretion and degradation rate of virtually all hormones; and the response of their target tissues to them. It can truly be said that no tissue or organ system escapes the adverse effects of thyroid hormone excess or insufficiency [7, 8, 9]. Thyroxine plays an important role by influencing the metabolic rate, carbohydrate metabolism, protein synthesis and lipid metabolism. it is evident that thyroid hormone is essential for glycolysis, gluconeogenesis and glucose absorption from gastrointestinal tract along with glucose turn over Estimation of thyroxine (T_4) level in normal and ketotic animal is an important observation in present research as thyroxine is a metabolic hormone and ketosis is a metabolic disorder of dairy animals [10, 11, 12, 13].

There have been varied observations on the correlation between thyroid hormone in blood and energy balance in high yielding dairy animal, with some reporting positive interaction [14], while others noting a negative correlation between T_3 and T_4 in the blood and milk production [7]. The mean value of serum thyroxine (nmol/L) in control group in present study was in line with the normal range of thyroxine level in blood of healthy cows [4, 12, 15]. Studies related to assessment of serum thyroxine level in conjugation with ketosis in buffaloes is still very limited. Earlier studies in cows [1, 4, 12, 15, 16] reported a significant reduction in serum thyroxine level. This variation may be attributed to different species, breed, nutrition, experimental planning, duration of experiment, stress factors, physiological and managemental condition etc. In present study, animals were in the early stage of ketosis so there was non-significant reduction occur in

thyroxine level whereas in other study animal may be in advance stage of ketosis so there was significant reduction occur in thyroxine level in ketotic animal.

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