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Effect of age and sex on thyroid hormone profile in Malpura sheep of Jaipur region

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Abstract
The objective of the current study was to evaluate the effect of age and sex on the thyroid hormonal profile in Malpura sheep of the Jaipur region. A total of 60 clinically healthy animals of both sexes (male and female) and three age groups, *viz*., 0-1 year, 1-2 years, and above 2 years were selected for the study. Blood samples were obtained from the jugular vein (10 mL each) under aseptic conditions, directly into serum-separating tubes (non-EDTA). The thyroid hormone profile (T_3 and T_4) was measured using a microplate-based (Thermo Scientific Multiskan FC) enzyme-linked immunosorbent assay (ELISA). A highly significant ($p<0.01$) effect of age and sex groups in Malpura sheep was observed on the Mean ± SE concentrations of triiodothyronine (T_3). Thyroxine, showed a highly significant ($p<0.01$) effect of age groups while these did not differ significantly ($p>0.05$) by sex. The increased concentration of serum thyroid profile, in the early stage (0-1 year) may be due to the enhanced physiological and metabolic activity during this period.

Keywords: Malpura sheep, thyroid hormonal profile, age, sex

Introduction
The Malpura breed of sheep is indigenous to Rajasthan and is one of the most important sheep breeds among eight breeds available in Rajasthan. This breed derives its name from the small town "Malpura" of the Tonk district of Rajasthan and predominates in Jaipur, Tonk, Sawai Madhopur, Ajmer, Chittorgarh and Bhilwara districts of Rajasthan. Ewes of this breed have good mothering ability and are having sufficient milk to sustain and accelerate the growth of their neonates. Lambs of this breed are quite suitable for fat lamb production. The animals are fairly well built and their face is of light colour with short and tubular ears. The fleece is white and extremely coarse. Malpura is a medium to heavy size breed and its adult average body weight is 40 kg in males and 30 kg in females (Kumar *et al*. 2008) [25].
Thyroxine (T_4) and triiodothyronine (T_3) are the hormones secreted by the thyroid gland. T_3 and T_4 levels in the serum must be determined in order to measure thyroid function. Thyroid hormones play a variety of roles in tissue differentiation, basal metabolic rate (BMR) growth, development, reproduction, and lactation, as well as lipid, carbohydrate, nitrogen (protein), and energy metabolism (Hoch, 1974; McDonald and Pineda, 1989; Swenson and Reece, 1996 and Sokkar *et al.*, 2000) [20, 26, 35, 34]. The actions are carried out by boosting the activity of certain enzymes involved in oxygen consumption. Thyroid hormone production by the gland and usage by target cells are regulated by free fractions rather than protein-bound parts (Bantle *et al.*, 1980) [5].
The age and sex-related studies on thyroid hormones and biochemical parameters may be useful in evaluating the adaptation and productivity of Malpura sheep in Rajasthan's severe environment. Taking into consideration all the information, the present study was carried out to evaluate the effect of age and sex on the thyroid hormonal profile in Malpura sheep of the Jaipur region.

Materials and Methods
A total of 60 clinically healthy animals of both sexes in three age groups, *viz*., 0-1 year, 1-2 years and above 2 years were selected for the present investigation.

The animals belong to the sheep and goat farm, LFC, PGIVER, Jaipur and the surrounding area of Jaipur region of Rajasthan.

Table 1: Experimental Design

Age Groups	Sex		Total
	Male	Female	
0 - 1 year	10	10	20
1 -2 year	10	10	20
Above 2 years	10	10	20
Total	30	30	60

Collection of blood sample

Blood samples were obtained from the jugular vein (10ml each) under aseptic conditions during the early hours (i.e. before feeding) from animals, posing minimum disturbance to the animals directly into serum separating tubes (Non-EDTA).

Hormones analysis

Thyroid Hormone Profile was estimated by Thermo Scientific Multiskan FC Microplate-based ELISA (Enzyme-Linked Immunosorbent Assay) using kits supplied by Auto Bio-Diagnostics Co. Ltd., Zhengzhou, China. The procedure was followed as suggested by the manufacturer.

Statistical analysis

Conventional standard statistical procedure was used to analyse the data for mean, standard error and ANOVA using SPSS 24th version and comparison of mean was tested using Duncan's multiple range test.

Results and Discussion

Triiodothyronine (T_3)

In the present investigation, overall serum Triiodothyronine concentration, irrespective of age and sex was 1.72±0.00 nmol/l in Malpura sheep. The observed Mean ± SE values of serum triiodothyronine (T_3) in sheep were more or less close to the range as illustrated by Kaneko *et al.* (1997) [21], Sharma and Kataria (2008) [33] and Gupta *et al.* (2010a) [17], although there was a variation which might be due to the difference in breed, age, sex and different environmental conditions.

The Mean ± SE values of serum triiodothyronine concentrations according to the age groups, irrespective of sex were 1.76±0.00, 1.69±0.00 and 1.72±0.00 nmol/l for 0-1 years, 1-2 years and above 2 years respectively; ranging between 1.69±0.00 to 1.76±0.00 nmol/l. A highly significant (p<0.01) effect of age was observed on serum triiodothyronine concentration in the present study. Triiodothyronine concentration was highest in the 0-1 years age group, followed by animals above 2 years of age and 1-2 years of age (lowest). Hence, there was no definite pattern noticed in the triiodothyronine levels as the age advanced. A similar pattern was reported by Gupta *et al.* (1998) [18] in Black Bengal bucks and Sharma and Kataria (2008) [33] in Magra sheep. However, they observed a non-significant (p>0.05) effect of age which was contraindicated to the present study. Eswari *et al.* (1999) [13] reported that the T_3 levels from 3rd month onward did not show any significant changes in Madras Red sheep. Pandey *et al.* (2014) [29] reported that serum T_3 declined progressively with the advancement of age in both sexes but did not differ significantly (p≥0.05) among the age groups in Marwari goats.

Azimzadeh and Javadi (2020) [4] reported that T_3 was significantly higher (p<0.05) in the over 2 years group compared with those under 2 years.

A similar result was observed by Habibabadi *et al.* (2000) [19] in Iranian goats, Firat *et al.* (2005) [15] in Sakiz sheep, Eshratkhah *et al.* (2010) [12] in Moghani sheep, Gattani and Sareen (2011) [16], Kassim *et al* (2018) [22] in Arabi sheep who reported that an increase in age results in significant (p<0.05) decreases in the plasma/serum concentrations of T_3. The higher concentrations of triiodothyronine observed in newborn lambs could be one of the adaptive mechanisms to overcome the stressful period by enhancing basal metabolism. It is now well accepted that T_3 is a more biologically active hormone rather than T_4 and T_4 is converted to T_3 at the cellular level of the site of action (Kohrle *et al.*, 1987) [24].

The Mean±SE values according to sex, irrespective of age were 1.71 ±0.01 and 1.73 ±0.01 nmol/l in male and female animals, respectively. The female animals had highly significant (p<0.01) serum triiodothyronine concentrations than the male animals.

Similar findings were reported by Pathak *et al.* (1989) [32] in buffalo calves, Eswari *et al.* (1999) [13], Celi *et al.* (2003) in cashmere-bearing goats and Pandey *et al.* (2011) [29] in Marwari goats who reported a significantly higher value of T_3 in females than in males. Sharma and Kataria (2008) [33] in Magra sheep observed non-significant (p>0.05) higher values of T_3 in females than males.

Differences in the estimation procedure, breed, season, and stage of maturity may be the cause of the observed variations in mean values obtained in the current study and those reported by previous researchers. Sex-related differences in thyroid hormones are also reported in other mammals which are the consequences of various actions of steroidal sex hormones.

Thyroxine (T_4)

In the present investigation, overall serum thyroxine concentration, irrespective of age and sex was 87.83±0.85 nmol/l in Malpura sheep. The observed Mean ± SE values of serum thyroxine in sheep were more or less close to the range as illustrated by Kaneko *et al.* (1997) [21] and Gattani and Sareen (2011) [16], although there was a variation which might be due to the difference in breed, age, sex and different environmental conditions.

The Mean ± SE values of serum Thyroxine concentration according to age groups, irrespective of sex were 95.45±0.27, 87.38±0.99 and 80.66±0.20 nmol/l for 0-1 years, 1-2 years and above 2 years, respectively; ranging between 80.66±0.20 to 95.45±0.27 nmol/l. A highly significant (p<0.01) effect of age was observed on serum thyroxine concentrations (T_4) being highest in the 0-1 years age group and lowest in animals above 2 years of age.

The statistical analysis of the data revealed that the mean serum thyroxine concentration decreased significantly (p<0.01) with advanced age. The higher concentration of thyroxine hormone observed in younger animals in the present study confirmed the earlier findings of Sharma and Kataria (2008) [33] in Magra sheep, Gattani and Sareen (2011) [16] in Magra rams, Pandita *et al.* (2016) [31] in Murrah buffaloes and Kassim *et al.* (2018) [22] in Arabi sheep. An increased thyroid hormone concentration was probably related to the elevation of metabolism rates in younger animals than older once (Carlos *et al.*, 2015) [8] and the biological activity needs more secretion of metabolic hormones such as T_3 and T_4.

This hormonal increase can be attributed to the fact that growth hormone is an anabolic hormone that stimulates

skeletal bone formation and elevation of protein synthesis and those metabolism processes are higher in younger animals and decline with the rise of age (Bassett *et al.*, 1971) [7].

The declining trend in the concentration of thyroxine with advancing age is also in agreement with the results of Eswari *et al.* (1999) [13], Neeru *et al.* (2001) [28] in sheep, Baruah *et al.* (1993) in goats, Khurana and Madan (1984) [23] in cows and Mehta and Varman (1983) and Agarwal *et al.* (2000) [1] in buffaloes.

Azimzadeh and Javadi (2020) [4] reported that T_4 was significantly (p<0.05) higher in the age over 2 years compared with those under 2 years. Pandey *et al.* (2014) [30] reported that serum T_4 declined progressively with the advancement of age in both sexes but did not differ significantly (P≥0.05) among the age groups in Marwari goats which was contrary to our findings. Eswari *et al.* (1999) [13] reported that levels of T_4 declined steadily up to 3 months of age indicating increased metabolism during growth. Higher thyroidal secretion rates in newborn animals had been reported to support the newborn animals to adopt the new extra-uterine environment (Anderson *et al.*, 1988) [3]. Thus, the significantly higher concentration of thyroxine in newborn lambs in the present study could be attributed to the higher thyroidal secretion rate in response to TSH to provide for stimulated oxidation in many cells and a defensive mechanism by the lambs after birth by increasing metabolic rate (Dickson, 1970) [11]. A reduced metabolic clearance rate could provide another possible explanation for elevated thyroxine concentration in animals during their early life (Khurana and Madan, 1984) [23]. The decline in the peripheral concentration of thyroxine in response to advancing age could be explained in view of the negative feedback phenomenon as a result of an already higher concentration of thyroxine in the blood.

The Mean ± SE values of serum T_4 according to sex, irrespective of age were 87.19±1.19 and 88.48±1.29 nmol/l in male and female animals, respectively. The female animals showed higher values of thyroxine hormone than the corresponding mean values in male animals, however, a non-significant (p>0.05) effect was observed. Similar findings were reported by Pandey *et al.* (2011) [29] in Marwari Goats.

The opposite findings were reported by Sharma and Kataria (2008) [33] in Magra sheep who observed a highly significant (p<0.01) higher concentration of thyroxine in females than in males.

Present findings are contrary to the study of Carlos *et al.* (2015) [8] in the Morada Nova sheep and Azimzadeh and Javadi (2020) [4] in Iranian Red sheep who reported that males had significantly higher values than females.

Sex-related differences are reported in other mammals and are referred to several actions by sexual steroid hormones: differences in total T_4 levels can be explained by oestrogen-reduced catabolism of thyroxine-binding globulin (TBG) (Ain *et al.*, 1987) [2], or androgen inhibition of the synthesis of TBG by the liver (Federman *et al.*, 1958) [14]. Moreover, androgens inhibit TSH secretion by the pituitary (Christianson *et al.*, 1981) [10].

Table 1: Mean ± SE values of serum triiodothyronine (T_3) and thyroxine concentration (nmol/l) in Malpura sheep with different age groups and sex

Mean Effects	Groups	No. of Observation	Triiodothyronine Mean ± S.E. (nmol/l)	Thyroxine Mean ± S.E. (nmol/ l)
Age	0-1 years	20	1.76 ^{c} ±0.00	95.45 ^{c} ±0.27
	1-2 years	20	1.69 ^{a}±0.00	87.38 ^{b} ±0.99
	Above 2 Years	20	1.72 ^{b} ±0.00	80.66 ^{a} ±0.20
Sex	Male	30	1.71 ^{a} ±0.01	87.19±1.19
	Female	30	1.73 ^{b}±0.01	88.48±1.29
Overall Mean		60	1.72±0.00	87.83±0.85

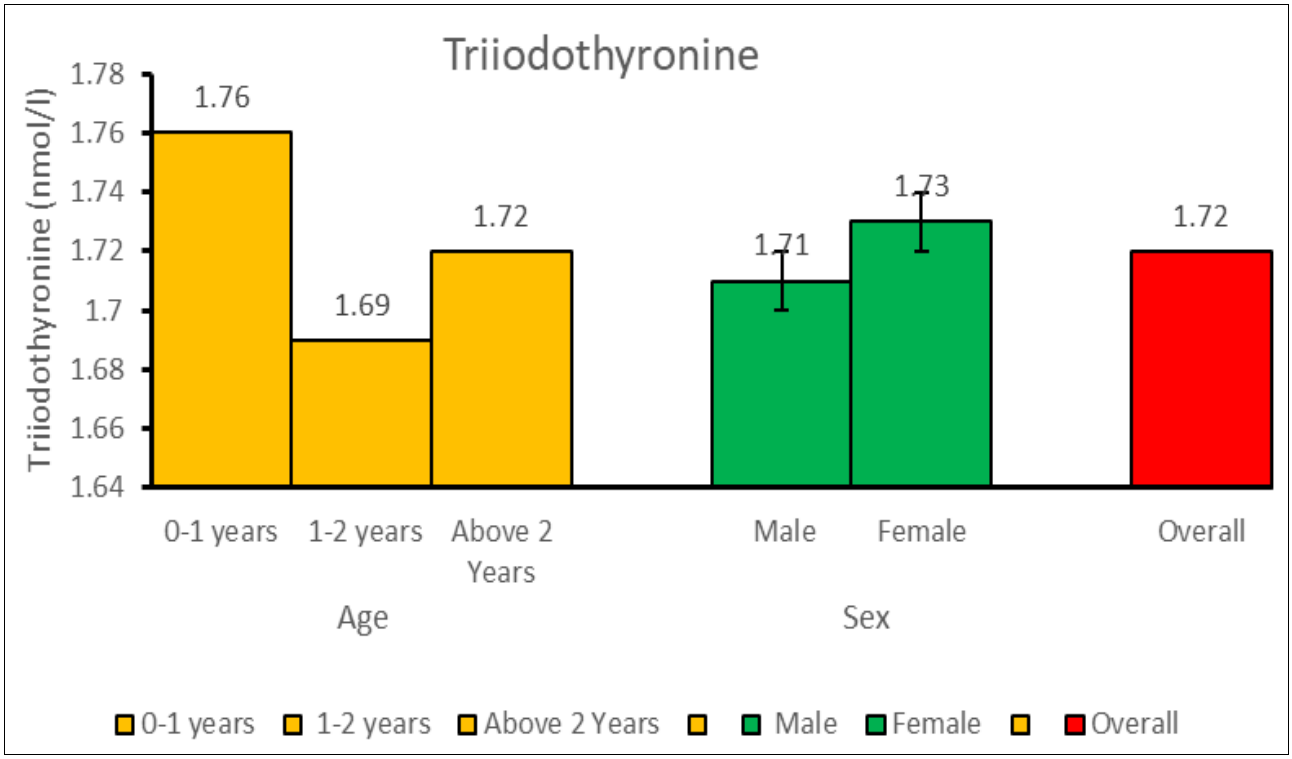

Fig 1: Effect of age and sex on serum triiodothyronine (T_3) concentration in Malpura sheep

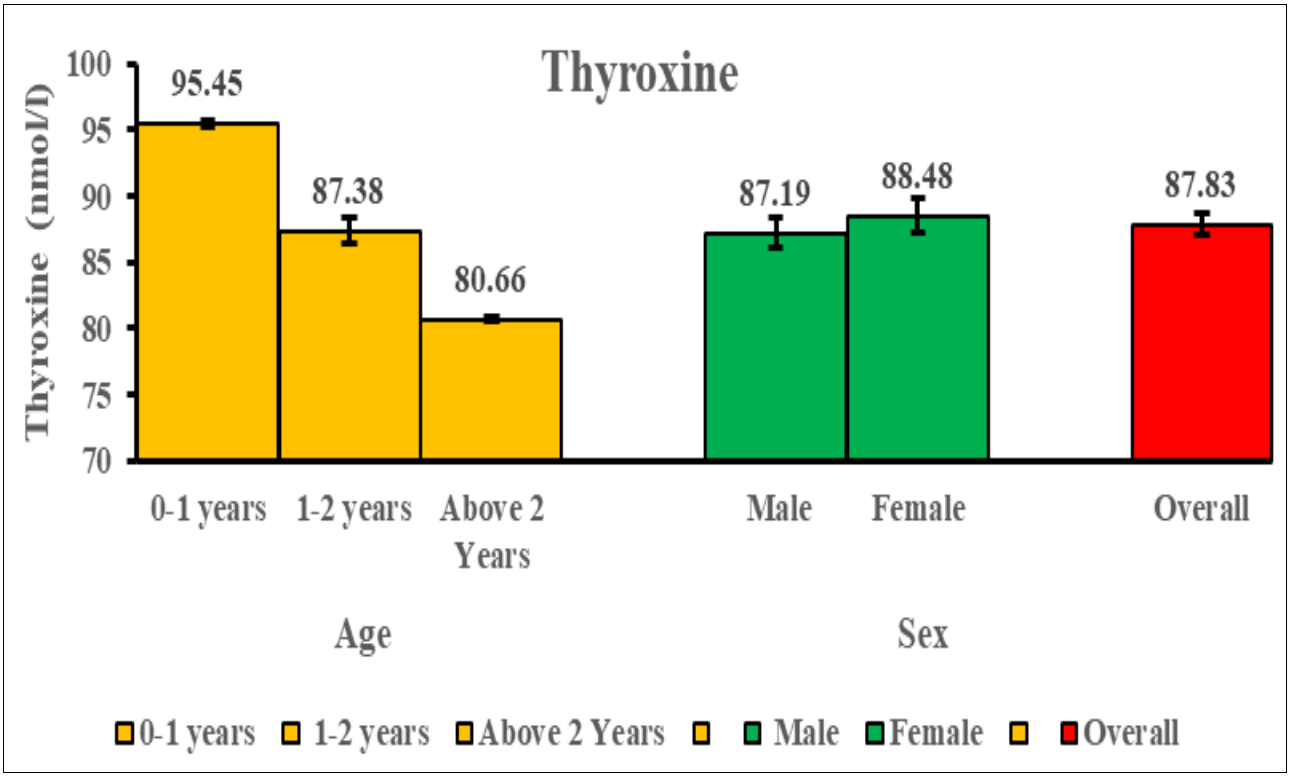

Fig 2: Effect of age and sex on serum thyroxine (T_4) concentration in Malpura sheep

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Conclusion
The study investigated the thyroid hormone profile in Malpura sheep, revealing significant age-related variations in triiodothyronine (T_3) and thyroxine (T_4) concentrations. T_3 levels were highest in the youngest age group (0-1 years), while T_4 concentrations peaked in the same group, showing a decline with age. Females exhibited higher T_3 levels compared to males, although no significant difference was observed in T_4 levels between sexes. These findings contribute to our understanding of thyroid hormone dynamics in sheep and underscore the importance of age and sex in hormonal regulation.

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