Int.J.Curr.Microbiol.App.Sci (2019) *8*(11): 1052-1061

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 8 Number 11 (2019)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2019.811.124

Hair Tissue Mineral Analysis during Preruminant, Transitional and Ruminant Age Groups in Reference to The Serum T3, T4 and Cortisol in Assam Hill Goat (*Capra hircus*)

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Keywords

Assam hill goat, Cortisol, Thyroid, HTMA, Hair, ICP-OES, Hair mineral

Article Info

Accepted:
10 October 2019
Available Online:
10 November 2019

A B S T R A C T

The present study was conducted in the Assam Hill Goat (*Capra hircus*) a local variety of Goat of Assam, to investigate the Hair Tissue Mineral patterns (estimated by ICP-OES) during three important metabolic stages *viz*, preruminant (0-3weeks), transitional (3-8weeks) and ruminant (above 8 weeks) age groups. Various hair minerals estimated in the present study were compared during the preruminant, transitional and ruminant age groups. The Ca content was found to be significantly lower in the preruminant age groups compare to the other age groups and maximum in the ruminant age groups. K was found to be synergistically related with Ca. Cr, Cu. Hair Na did not show any significant changes during the three age groups. Fe, K, Mg and Zn content of hair of Assam Hill Goat showed significantly higher values in ruminant age groups and the mean concentration of these elements in the hair showed an increasing pattern with the advancement of age. Various important mineral ratios were compared in the present study. It was found that Na:K, Na:Mg were found to be significantly lower in ruminant age group than preruminant and transitional age groups and with advancement of age there was decrease in the values of ratio. The serum T_3 and T_4 and cortisol as found to be significantly higher during preruminant age group than transitional and ruminant age groups. Ratios like Na:Mg, Na:K and Ca:K can be correlated with the serum cortisol and thyroid hormone respectively may be indicative of the activity of the adrenal and thyroid gland as well as the metabolic activity. The mean values of Ca:K, Zn:Cu and Fe:Cu showed an increasing pattern with the advancement of age and Ca:Mg ratio showed lower values in ruminant age groups than preruminant and transitional age groups.

Introduction

Hair is a dead keratin and contains a mineral fraction. These mineral fractions get deposited over a period of time during the growth phase of the hair.

The elemental concentration in hair is more meaningful as it provides historical information about the metabolic status on the elemental concentration in the body as well as nutritional condition over a long period of time, unlike blood or urine, which merely indicates the body conditions only on the date of collection (Laker, 1981 and Ahmad *et al.,* 2013). Trace element concentration is higher in hair compared to blood or urine, and so it better reflects the content in the body than the other biological materials.

Hair analysis provides information about intracellular accumulations of trace elements and has been used to evaluate the trace element status in the body (Ozmen *et al.,* 2013). So Hair tissue mineral analysis has been found to be an excellent tool for monitoring mineral status in an animal and metabolic status, glandular activity etc by analyzing the concentration & ratios of various trace minerals. As the minerals are interrelated and interact with each other and maintain a homeostasis.

So the establishment of mineral concentration and range can be helpful to identify the metabolic deficiency and chronic diseases.

Various ratios like Ca:K, Na:K, Ca:K, Zn:Cu, Na:Mg, Ca:Mg, Fe:Cu are important indicating the glandular activity and general metabolic function. So the present investigation is aimed at studying the general pattern of change in the hair mineral during the three important metabolic stages (preruminant, transitional and ruminant) and various mineral ratios in reference to the serum thyroid and cortisol hormone particularly in the Assam Hill Goat being a local importance.

Materials and Methods

Location of study

The present study was conducted for the investigation of hair tissue mineral change pattern during preruminant, transitional and ruminant stage of development of Assam Hill Goat.

For this investigation the Assam Hill Goats were identified from in and around the Guwahati city of Assam, as well as the samples were also collected from the Goat Research station Burnihat, Assam Agricultural Universirty, India. Hormonal analysis was performed in the Veterinary Physiology Department, College of veterinary Sciences, Khanapara AAU, India, and mineral estimation study was performed at Tejpur University, Tejpur, Assam.

Selection of animals and sampling

The hair samples of Assam hill goat were collected randomly from leg, head, back region, irrespective of sex, coat colour during preruminant age groups (0-3weeks of age), Transitional (3-8 weeks) and ruminant (8 weeks above). Samples were collected from 24 numbers animals with 8 numbers in each age groups. From those same animals blood samples were also collected for serum.

Collection and preservation of samples

Approximately 4-5 gm hair samples were collected from the rump and leg, back, and head region irrespective of sex and body coat color with scissors and washed. Kept in plastic pouch. The blood samples were collected in clot activator vial and then serum samples

were separated and kept in -20ºC (deep freeze) till the analysis for hormone.

Sample analysis

Hair sample of 0.3 gm was treated with 3 ml concentrated nitric acid (69%). The mixture was then kept in the hot water bath for 15 minutes (until the hair gets completely dissolved) at 60ºC. Solution was then diluted with 30 ml ultrapure water. After that the content was filtered through the whatman filter paper.

The clear liquid aliquot was taken to the Tezpur University (SAIF), Tezpur, Assam for the mineral estimation. Hair samples were analyzed for Ca, Cr, Cu, Fe, K, Mg, Mn, Na, Zn by Inductively Coupled Plasma –Optical Emission Spectroscopy (ICP-OES) at SAIF, Tezpur University Assam, India.

To estimate the serum T_3, T_4 and cortisol, 5ml of blood samples were collected from each animal belonging to different experimental groups. Serum was separated and kept in the -20 ºC.

The serum samples analyzed for serum T_3, T_4 and cortisol by the standard procedure of Radioimmunoassay (RIA) using RIA kits (I^{125} labeled) supplied by Immunotech (Beckman Coulter Inc.)

Statistical analysis

Various parameters were analyzed by Biostatistical design and Analysis using R.A Practical Guide, Willey Blackwell Publication, West Sussex, UK by Logan (2010).

Ethical Approval: The Research work was carried out as per the approval of the Institutional Animal Ethics Committee, Approval No: 770/ac/CPCSEA/FVSc/AAU/ IAEC/16-17/373 dated 30.07.2016, Assam Agricultural University: Khanapara, Guwahati-781022)

Results and Discussion

The mean values of hair Ca showed that with the advancement of age the calcium fraction of hair increased which might be due to increase in the rate of Ca metabolism, as calcium is the structural mineral for bones. Findings were supported by Mujeeb *et al.*, (2017) in human. In the present investigation the higher potassium level during ruminant age groups than the preruminant and transitional group may be due to synergistic relation of potassium and calcium (Wilson 2017 in human). Higher values of iron content of hair during ruminant age groups can be supported by Combs *et al.*, (1982) in young cattle. Rashed *et al.*, (2002) reported that Fe concentration in goat hair was 45 µg/g. The variation in the hair Ca and Mg in the present investigation might be due to change in the metabolism pattern, diet (Combs *et al.*, 1982 and Anke (1966) or hair color (Fisher *et al.*, 1985). The mean concentration of Chromium, Copper and sodium content during preruminant, transitional and ruminant age groups were 0.046±0.01, 0.034±0.002, 0.047±0.008 and 0.15±0.021, 0.11±0.008, 0.176±0.031 and 25.0±0.84, 24.57±0.91, 24.78±0.969 mg/L, respectively, ($P>0.05$) (Table 1). The copper fraction of hair was maximum during higher age groups (ruminant) compare to preruminant and transitional. Which might be due to increase in the bone calcium with the age advanced and as Cu is essential for fixing the Ca to the bone which was also supported by Wilson (2017) in human. The concentrations of copper in hair reported by various authors were Holasova (2017) reported 10.1 mg/kg dry matter in South American camelids. Sokola *et al.*, (2009) reported 5.3–10.3 mg/kg in sheep wool and Szczegielniak *et al.*, (2014) reported 10.2–

32.0 mg/kg in cow hair. In this investigation the hair copper concentration could be compared with the cow hair and bit higher than the sheep hair. However variations might be due to diet (Perez *et al.,* 2000). Kellaway *et al.,* (1978) concluded that Cu levels in hair were a sensitive index of Cu status of body. However Holasova (2017) also reported that the hair copper concentration did not vary with the age sex, and color in American camelidaes. Combs *et al.,* (1982) reported higher Na content in younger cattle. Anke *et al.,* (1965) reported that Ca, Mg, K and Na content of hair varied with coat color in cattle. From the above study it was concluded that the variation in the hair Ca, Mg, K and Cu content in the present study might be due to coat color, interaction with other element, physiological state and supplementations.

The increase in the Zn content in the higher age groups might be suggestive of increase in the degree of keratinization and mineralization. Hair Zinc content reported by various authors such (Holasova 2017) reported 134.4 mg/kg dry matter in American camelids. Sokola *et al.,* (2009) reported 75.0 to 88.8 mg/kg dry matter in sheep wool; Szczegielniak *et al.,* (2014) reported 125.7–427.4 mg/kg dry matter in cow hair. Pavlata *et al.,* (2011) reported 97.9 ± 10.1 mg/kg dry matter in goat hair. Though Klevay *et al.,* (2004) have reported that the Zn content of hair depends on the species. (Miller 1970, Deeming and Weber 1977 and Combs *et al.,* 1983) demonstrated the correlation of hair Zn with dietary Zn intake.

Significantly higher Manganese content during transitional and ruminant age groups of Assam Hill Goat in the present study compare to preruminant age, i.e. earlier days of life may be suggestive of increased degree of keratinization during higher age groups. Holasova (2017) reported the hair manganese of 12.62 mg/kg dry matter in American camelids. Sokola *et al.,* (2009) reported 3.4 to 22.9 mg/kg dry matter in sheep wool; Szczegielniak *et al.,* (2014) reported 3.8–20.0 mg/kg dry matter in cow hair. (Holasova 2017) reported that age sex color had significant affect on the hair manganese content. Rashed *et al.,* (2002) reported the Mn content of goat hair was 879 µg/g. The higher values of Fe and Mn with the advancement of age might be due to growth and development of hair follicle as manganese and iron are important element in functioning and development of hair follicle which was also supported by Rashed *et al.,* (2002) in goat.

The higher values of Ca, Mg, Mn and Mg in the present study during the advanced age groups (transitional and ruminant) were also supported by (Clauss and Dierenfeld, 1999). (Onwuka, 2000) reported that Mg content was most abundant element compare to Zn and Cu in West African dwarf goat.

Battini *et al.,* (2015) reported the variation in the trace mineral content in rough and smooth haired goat. Szigeti *et al.,* (2015) reported variation amongst the breed of cattle in the hair mineral concentration. Combs *et al.,* (1982) reported that hair mineral as an indicator of mineral status in cattle and mineral content of hair was affected by season, breed, and hair color within and between breeds, sire, and age and body location. Bhattacharya *et al.,* (2004) stated that elemental concentration in yak hair varied due to location but not due to age and sex. Holasova (2017) reported the variation in the hair mineral content in lama and Alpaca and also stated the mineral content varied with coat color. Wilson (2016) reported that the values may vary with breed and species. From the above review and the present investigation it was understood that the variation of mineral content of hair may be due to species, breed, sex, age, color, metabolic status and supplementation. In the present study the

change pattern of hair minerals during preruminant, transitional and ruminant age groups which might be due to the change in the physiological status of Assam Hill Goat. Gabryszuk *et al.,* (2000) also reported variation in hair mineral with the change in physiological status in sheep. The values for hair minerals need to further evaluated considering the various factor like sex, age, color, metabolic status and supplementation in order to establish the standard values, which shall reveal a numbers of information.

Higher T3 and T4 during earlier days of life in Assam Hill Goat and with the shift of ages from preruminant to transitional to ruminant there was fall in the hormonal concentration may be due to high demand of energy and metabolic rate or may be due to the change in the metabolic pattern and physiological state in the present study.

This was supported by Todini *et al.*, (2007) in small ruminants and Polat *et al.,* (2014) in white goat. Valavi *et al.,* (2017) reported age dependant change in the serumT_3 and T_4 concentration in the Saanen Goat.

Significantly higher cortisol level in the present study during preruminant age groups might be an indicative of high metabolic stress. Olsson and Hydbring- Sandberg (2011) reported the serum cortisol level between 17 and 49 nm/L in goat exposed to fear-eliciting stimuli. Minton *et al.,* (1995) found that the plasma concentration of cortisol hormone in Merino sheep, without imposing any kind of stress, was 20 ng/mL.

Andersen *et al.*, (2008) measured the serum cortisol level in goat between 2-10ng/ml exposed to social instability. Nwe *et al.,* (1996) measured the serum cortisol in goat exposed to transport stress was 42-169 ng/ml. Kjoren *et al.,* (2012) measured the basal cortisol level in goat varied from 4.0-64.8 nm/L

With the age advanced, there was adaptation in the level of serum cortisol over period of time during transitional and ruminant age groups with lower values of cortisol may be due to adaptation of animal to metabolism. Todini *et al.,* (2007) reported variation in the serum thyroid concentration during various physiological stages in small ruminants. Alves *et al.,* (2016) also reported that the adaptation over a period of time influence the serum cortisol level Santa Ines lambs.

As minerals in the body are maintained in a homeostasis in interaction with other antagonistic and synergistic elements. So some important mineral ratios were studied and compared with serum thyroid and cortisol hormone in the present investigation. The values of Na:K ratio was found to be decreased with the age advanced during preruminant, transitional and ruminant age group and higher values were observed during preruminant age group. Wilson (2016) reported the Na:K ration in goat hair ranged from 0.35-0.40, as goat being fast oxidizer.

The change pattern of Na:K ratio in the present investigation during preruminant, transitional and ruminant age groups could relate with the serum cortisol levels.

It was found that with the higher ratios of Na:K during preruminant age group there was higher values of serum cortisol. Findings were in consistence with the McEvoy (2011) and Watts (2010) in human as Na:K ratio was also called as adrenal ratio. The ratio of Ca:K and Zn:Cu during preruminant, transitional and ruminant age groups were 0.47±0.013, 0.57±0.034 and 0.56±0.06 and 7.18±0.65, 8.67±0.42 and 9.11±0.90 respectively. Wilson (2016) stated that increase in the Ca reduces the thyroid activity and increase in the K increases the thyroid secretion so the ratio of Ca: K maintains in homeostatic ratio and control the serum thyroid level (thyroid ratio). In the present investigation serum T_3 and T_4

concentration during preruminant, transitional and ruminant age groups showed a opposite relation with the Ca:K ratio. This was supported by Wilson (2016), McEvoy (2011) and Lydia, (2016) in human. The mean values of Zn:Cu showed an increasing pattern with the advancement of age in the present study. With the increase in the ratio of Zn: Cu there was fall in serum thyroid level (Table 2 and 3).

Table.1 Minerals of hair during preruminant, transitional and ruminant age groups of Assam hill goat by ICP-OES

	PRERUMINANT	TRANSITIONAL	RUMINANT
Parameters	Mean±SE (mg/L)	Mean±SE(mg/L)	Mean±SE(mg/L)
Ca	29.17±0.808	35.793±2.787	36.92±2.814
Cr	0.046±0.0109	0.034±0.0024	0.047±0.00878
Cu	0.152±0.0218	0.117±0.0083	0.176±0.031
Fe	1.2388^{a}±0.097	1.103500^{a}±0.032	1.5216^{b}±0.082
K	61.910±0.864	63.618±5.59	67.31833±3.68
Mg	8.13±0.438	10.0±0.481	10.72±1.264
Mn	0.0881^{a}±0.0051	0.17^{b}±0.0298	0.17^{b}±0.0164
Na	25.0±0.843	24.57±0.910	24.78±0.969
Zn	1.024^{a}±0.056	1.011^{a}±0.065	1.473^{b}±0.132

Table.2 Ratios of different mineral of hair during preruminant, transitional and ruminant age groups of Assam Hill goat

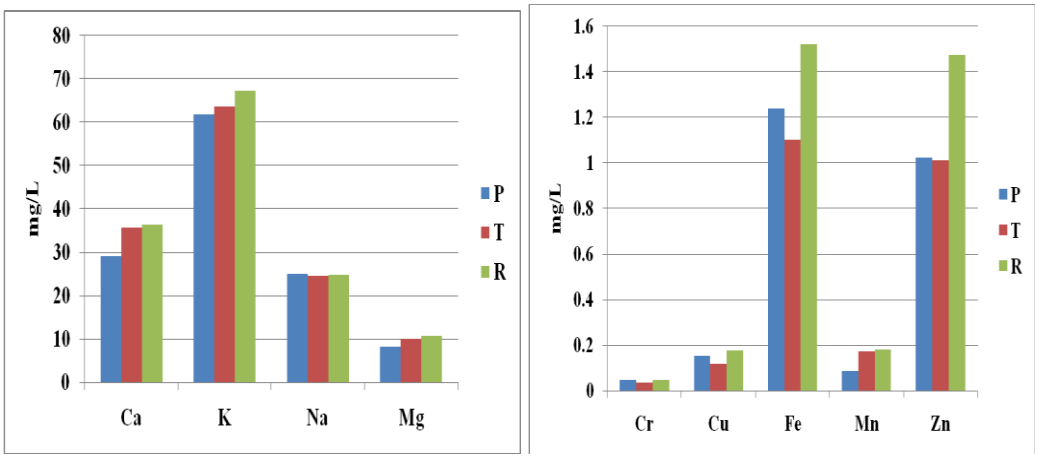
	PRERUMINANT	TRANSITIONAL	RUMINANT
Parameters	Mean±SE	Mean±SE	Mean±SE
Na:K	0.404±0.016	0.397±0.029	0.374±0.026
Ca:K	0.471±0.013	0.571±0.034	0.565±0.061
Zn:Cu	7.183±0.653	8.670±0.422	9.115±0.905
Na:Mg	3.13±0.258	2.47±0.113	2.47±0.287
Ca:Mg	3.63±0.199	3.57±0.203	3.65±0.481
Fe:Cu	8.55±0.684	9.565±0.519	10.10± 1.728

Table.3 Serum T_3, T_4 and cortisol during Preruminant, Transitional And Ruminant age groups of Assam Hill Goat

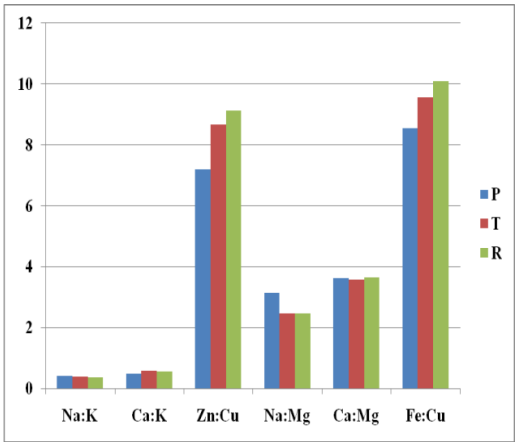
	PRERUMINANT	**TRANSITIONAL**	**RUMINANT**
Parameters	Mean±SE (nMol/L)	Mean±SE (nMol/L)	Mean±SE (nMol/L)
T_3	3.82^{a} ±0.59	3.04^{a}±0.511	2.77^{b}±0.29
T_4	101.76^{a} ± 18.014	60.76^{b} ±9.796	40.48^{c} ±3.274
Cortisol	35.45^{a} ± 0.64	19.94^{b} ±0.321	18.73^{b} ±0.560

Means within the same row in each item within each group carrying different superscripts are significantly different at ($p<0.05$)

Graph.1(a,b) Graphical representation of mean values of different hair minerals during preruminant, transitional and ruminant age groups of Assam Hill goat by ICP-OES



Graph.2 Graphical; representation of various mineral ratios of hair during preruminant, transitional and ruminant age groups of Assam Hill Goat



P=Preruminant , T= Transitional, R= Ruminant , Ca= calcium, K= Pottassium, Na= Sodium, Mg= Magnesium, Cr= Chromium, Cu= Copper, Fe=Iron, Mn= Manganese, Zn= Zinc, Magnesium, Cr= Chromium,

As Zn and Cu both interact with serum K level and influence the serum thyroid level. In the present study increased ratio of Zn:Cu with the age might be due to sex steroid dominance which was supported by Watts (2010) in human. As Zn and Cu are essential for the maintenance of serum steroid hormone (Watts, 2010).

Higher values of Na:Mg ratio during in preruminant age groups of goat than transitional and ruminant age groups may be indicative the higher adrenal activity during preruminant age groups with higher cortisol level. Observations were also supported by Watts (2010) and Lydia (2016) in human. The ratio of Ca:Mg and Fe:Cu during preruminant, transitional and ruminant age groups were 3.63 ±0.199, 3.57±0.203 and 3.65±0.481 and 8.55 ±0.684, 9.565±0.519 and 10.10± 1.728 respectively. The Fe: Cu ratio showed an increasing pattern indicated that with the

advances of age there was increase rate of cellular metabolic activity and accumulation of free radicals as reported by Watts 2010 in human.

In the present study the change pattern of hair tissue mineral and its change pattern during three metabolic phase of Assam Hill Goat viz, preruminant, transitional and ruminant age groups can be compared. As the minerals are maintained in the body in homeostasis in interaction with hormones and metabolism. The sodium, potassium, calcium, magnesium their values and ratios can be used as an indicator of stress, metabolic deficiencies' considering the serum thyroid and adrenal hormone, as being the important metabolic hormones.

The hair Zn, Cu, Fe can be considered to evaluate the hair keratinization, coat colour and free radical damage of body. The standard mineral ratios can be useful for identification of metabolic disturbances, to overcome the nutritional deficiencies, area specific supplementation of mineral to the animal nutrition.

Acknowledgement

The authors is thankful and duly acknowledge the Incharge SAIF, Tejpur University, Assam, INDIA, for proving the facility of ICP-OES, the Head of the Department of Anatomy & Histology, Dean Faculty of Veterinary Science, Khanapara, Director PGS, C.V.Sc, AAU, Khanapara.

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How to cite this article:

Sukanta Das, Kabita Sarma, Mohan Bhattacharya, Abdus Saleque and Shantanu Tamuly. 2019. Hair Tissue Mineral Analysis during Preruminant, Transitional and Ruminant Age Groups in Reference to The Serum T3, T4 and Cortisol in Assam Hill Goat (*Capra hircus*). *Int.J.Curr.Microbiol.App.Sci.* 8(11): 1052-1061. doi: https://doi.org/10.20546/ijcmas.2019.811.124