Sheep breed type effects on plasma thyrotropin, thyroxine and testosterone in growing ram lambs under hot climate

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Abstract
The lack of knowledge about different physiological responses among indigenous sheep breeds raised in arid hot regions promoted the researcher to monitor a metabolic and a reproductive hormone in male lambs of two common breeds in Saudi Arabia. Forty eight growing male lambs (age 3-5 months) were grown in a private farm for fattening purpose. The animals were of two known breeds commonly raised in Saudi Arabia, namely Najdi and Noemi. Twenty four Najdi and 24 Noemi lambs were utilized in the study during hot months (June-August). Jugular blood samples were collected for eight consecutive weeks. Plasma was harvested and thyrotropin (TSH), thyroxine (T4) and testosterone (T) were determined. Significant (P<0.05) increases were found in TSH, T4 and T in Noemi (1.54 m IU/l, 11.91 µg/dl and 6.25 ng/ml, respectively) compared to Najdi (1.39 m IU/l, 7.55 µg/dl and 3.39 ng/ml, respectively) lambs. The week to week changes in the levels of the three hormones were not statistically significant (P>0.05). The increases in the three hormones in Noemi males paralleled the concurrent increases in body growth rates (174 vs 139 g/hd/day for Noemi vs Najdi). In conclusion, within a species physiological differences are worth to be monitored for managerial purposes.

Key words: Breed-type, hot climate, rams lambs, testosterone, thyrotropin, thyroxine.

Introduction
To our knowledge, there are very rare, if none published data on sheep breed differences of physiological parameters. Hot climate might modulate these physiological responses. At birth, Blackface lambs had higher 3,5,30-triiodothyroinone (T3) and thyroxine (T4) levels than Suffolk lambs and this was correlated with higher body temperature and better thermoregulatory ability 1. Merino lambs aged 2 to 3 days, submitted to cold stress, showed a stronger increase of T3 and T4 levels compared with Romney-Marsh lambs2. Lamb breeds that are usually reared under extensive conditions (hill regions) have an improved thermoregulation than those reared intensively in lowland. This is partly related to birth coat characteristics, accompanied by higher T3 and T4 concentrations (important for endogenous heat production and hair growth) in hill than lowland lambs 3. Assaf ewes had higher serum T4 concentrations than Rasa Aragonesa and Merino ewes, which was associated with differences in wool growth rate 4. Higher plasma T4 levels in Suffolk ewes than in Gulf Coast native ewes in the US were shown to be positively related to larger body size and enhanced growth potential 5. Higher levels of T3 and T4 in ram lambs have been associated with higher prolificacy of the Outaouais breed compared with the Suffolk breed (lower prolificacy) 6. The decline in serum T4 levels induced by feed restriction was greater in crossbreed ewes than in native Indian sheep 7. It has been shown a strong relation between thyroid hormone and testosterone levels. Therefore, the objective of the current study aimed to highlight the differences in a metabolic and androgenic hormone in the two most commonly demandable sheep breed in Saudi Arabia.

Materials and Methods

Animals and management: Forty eight weaned and healthy Najdi (n = 24) and Noemi (n = 24), 3-5 months age male lambs were housed in four open semi-shaded yards (12 animals /yard). Animals were raised in a private farm near the university. Feed was offered at 300 g pelleted ration (16% crude protein) per head per day in addition to alfalfa hay as free choice. Also, clean tap water and balanced mineral licks were offered as free choices. Animals were subjected to the mandatory schedule of vaccination. Experiment was conducted during the hot months (June-August) of 2010 summer.

Blood sampling: Jugular blood samples were collected once a week in Vacutainer heparinated tubes. Samples were transported in an ice box to the physiology laboratory within an hour and centrifuged (5°C) at 3000 rpm/ 15 min. Plasma was aspirated in clean labeled tubes and stored frozen (-20°C) until hormones were assayed.

Hormone assays:
Thyroid stimulating hormone (TSH): An ELISA commercial kits (Human, Wiesbaden, Germany) was used. Based on a classical sandwich method, the plate was coated with streptavidin. At first incubation step, specimens, calibrators or controls, enzyme-conjugate (peroxidase-labeled anti-TSH) and a second biotinylated monoclonal anti-TSH were mixed to form the sandwich complex. This binds to the surface of the wells by the interaction of biotin with the immobilized streptavidin. At the end of the incubation, excess enzyme conjugate and monoclonal antibodies...
were washed out. Tetra methyl benzidine (TMB)/Substrate ($H_2O_2$) was added for 15 min and enzyme-substrate reaction was stopped ($0.5 \text{ M } H_2SO_4$). The absorbance was read at 450 nm wavelength. The amounts of free TSH were proportionally related with the color intensity. The intra and inter-assay coefficients of variation were 3.2 and 4.3%, respectively.

**Thyroxine (T4):** Total T4 was measured in plasma samples according to Chopra et al. by competitive ELISA commercial kits (Human, Wiesbaden, Germany). The plates were coated with anti-T4 (raised in sheep). The intensity of the developed color was inversely related with the concentration of the free T4 in samples. The intra and inter-coefficient of variations were 4.2 and 5.1%, respectively.

**Testosterone (T):** According to the method of Kicklighter and Norman, testosterone levels in sheep plasma were measured in a simple solid phase competitive ELISA by commercial kits (Human, Wiesbaden, Germany). Also the free hormone concentrations were inversely related to the color intensities. The intra and inter-assay coefficient of variations were 3.4 and 4.1%, respectively.

**Statistical analysis:** Data were analyzed by the GLM – ANOVA for repeated measures on the same animal. Differences between breeds were tested by t-test. The following linear model was applied:

$$Y_{ijk} = \mu + B_i + W_j + e_{ijk}$$

where $Y_{ijk}$ = observation on $ijk^{th}$ hormone value, $\mu$ = overall mean, $B_i$ = fixed effect of $i^{th}$ breed type, $W_j$ = fixed effect of $j^{th}$ week of sampling and $e_{ijk}$ = random error.

**Results**

As illustrated in Fig. 1 there was a significant (P<0.05) elevation in the circulating TSH in Noemi (1.39 ± 0.07 mIU) compared to Najdi (1.54±0.10 mIU) ram lambs. Within the breed, there was also found a decrease with no statistical differences by the advancement of week of sampling (Fig. 2).

Thyroxine (T4) profile (Fig. 3) exhibited precocious increase (P<0.05) in blood plasma of Noemi (11.91±1.57 µg/dl) compared to Najdi (7.55±1.85 µg/dl) lambs. The week effect on T4 levels (Fig. 4) was significant (P<0.05), since the mean T4 levels started to decline at the fourth week of sampling reaching about 57-77% of their original values and continued to decrease thereafter until week 6.

Mean testosterone (T) concentration (Fig. 5) was statistically higher (P<0.05) in Noemi (6.25±1.21 ng/ml) than in Najdi (3.38±1.42 ng/ml) lambs. There was no obvious difference in T levels by the progression of sampling weeks, even though there appeared to exist a tendency of declining in T levels by time progress (Fig. 6).

**Discussion**

Thyrotropin (TSH) and total thyroxine (T4) levels were higher in Noemi [1.39 mIU (TSH) and 11.91 µg/dl (T4)] than in Najdi [1.54 mIU (TSH) and 7.55 µg/dl (T4)] ram lambs. The increases in such metabolic hormones coincided with the significantly higher daily growth weight in Noemi than in Najdi lambs. There might also exist some effect due to body coat black color in Najdi breed, which may constitute a more thermal burden on the body absorbing
In the current study, the experiment has been conducted during aged 2 to 3 days subjected to cold stress, showed stronger wool growth) in hill than in lowland lambs. Also, Merino lambs the birth coat accompanied by higher thyroid hormones in lowland. They attributed this response to the characteristics of improved thermoregulation compared to those reared intensively usually reared under extensive conditions (hill regions) have an

Moreover, in adult sheep, more than 99.9% of T4 and 99.5% of T3 be the main (77%) secretory product from thyroid gland.

Appropriate thyroid gland function and activity of thyroid hormones (T3 and T4) are considered crucial to sustain the metabolic and nutritional status of the animals. Since T3 and T4 are both used as biomarkers for basal metabolism and they are thermogenic hormones, the decrease of the circulating T4 in Najdi compared to Noemi lambs might explain the lower growth rate in Najdi (139±10 g/hd/day) than in Noemi (174±12 g/hd/day) growing lambs in the current study. At the tissue level, thyroid hormones act on many different target tissues, stimulating oxygen uptake and heat production in every cell in the body. The overall effects for T3 and T4 are to increase the basal metabolic rate, to make more glucose available to cells, stimulate protein synthesis, increase lipid metabolism and stimulate cardiac and neural functions. Thyroxin was found to be the main (77%) secretory product from thyroid gland. Moreover, in adult sheep, more than 99.9% of T4 and 99.5% of T3 disperse in blood bound to plasma proteins. Only the free hormone is responsible for the biological activity and protein-bound hormones function as a promptly utilizable storage, delaying the effects of decreased thyroid secretion, as well as acting as a buffer against sudden increases in thyroid’s secretory activity.

Dwyer and Lawrence demonstrated that lamb breeds that are usually reared under extensive conditions (hill regions) have an improved thermoregulation compared to those reared intensively in lowland. They attributed this response to the characteristics of the birth coat accompanied by higher thyroid hormones concentrations (important for endogenous heat production and wool growth) in hill than in lowland lambs. Also, Merino lambs aged 2 to 3 days subjected to cold stress, showed stronger increase of T3 and T4 levels compared with Romney-Marsh lambs.

In the current study, the experiment has been conducted during the hot months of June–August which are characteristic of the longest day light in the year. This coincides with Todini et al. who found a strong relation between the long day and the concentration of circulating T4 in Alpine and Saanen bucks. The slight decrease in TSH and T4 by time progression in the present study doesn’t interfere with this finding since the effect of the high thermal stress during the months of July and August (high ambient temperature and low relative humidity) in the Arabian Desert surpassed the effect of the longer day length during this period on suppressing the release of thyroid hormones.

Mean plasma testosterone concentration exhibited about 185% higher levels in Noemi than in Najdi lambs. This coincided with the earlier puberty and better semen quality in Noemi than in Najdi rams. Breed type effect was studied in ovine by Fallah-Rad and Connor who found a significant testosterone elevation in serum of Outouais compared to Suffolk ram lambs, which paralleled the concentrations of T3 and T4 in both breeds. It is believed that thyroid hormones accelerate differentiation of Sertoli cells. Moreover, a critical role of thyroid hormones in the testicular development of neonatal rats has been demonstrated. At the testicular level, thyroid hormones are influential during the perinatal period which coincides with the development of the rat gonads.

High levels of thyroid hormones can have potential impacts on the functional Sertoli cells. These effects can be either directly or indirectly. Direct influence of Sertoli cells on the production of androgens by Leydig cells and synthesis of androgen-binding protein (ABP) by Sertoli cells maintains testosterone concentration in seminiferous tubules at high level. There is an intimate association between thyroid hormones, testosterone and the GH-IGF-I axis. Due to the increase of heat stress by time progression during carrying the experiment, there appeared that testicles were exposed to excess heat stress which impedes the testicular activity. This resulted in a decline in T levels especially in Najdi males.

**Conclusions**

Raising sheep under hot climates requires intensive multidisciplinary studies to highlighten various mechanisms regulating adaptation to such a harsh condition.

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**References**


