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The influence of experimental hypothyroidism on hepatic and renal function in rams in an arid tropical environment

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ABSTRACT

Crop residues are rich in thiourea content, on account of the indiscriminate use of thiourea as a fertilizer. It is interesting to investigate the impact of thiorea feeding on liver and kidney functions in adult sheep. Experimental hypothyroidism was induced by *per os* feeding of thiourea to nine adult Marwari rams. Prior to the start of the trial, plasma levels of thyroxin (T4), thyroid stimulating hormone (TSH), alkaline Phosphatase (ALP), Aspartate aminotransferase (AST), alanine amminotransferase (ALT), glucose, protein, A/G ratio, BUN and creatinine were assessed to establish control values. The clinical profile of the rams and evaluation of the above parameters were conducted on Days 3, 5 and 7 post thiourea feeding. At the end of the experiment a decrease in respiration, heart rate, temperature and feed intake were observed. A significant fall in T4 and glucose was observed on day 7, whereas a significant increase in TSH, ALP, AST and ALT were observed. The elevation in protein, BUN and creatinine concentrations was not much significant. The altered hormone profile revealed the hypofunction of the thyroid gland. The increased concentration of enzymes revealed bile duct hyperplasia, damage to hepatocytes and the biliary network. Further, a shift in metabolic profile suggested low energy intake and depressed growth. In conclusion, thiourea feeding induces hypothyroidism, generalized oxygen starvation at tissue level and disturbs hepatic and renal functions.

Key words: thiourea, hypothyroidism, thyroxine, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase

Introduction

The thyroid gland is an important endocrine gland regulating metabolism in animals. Its secretions [thyroxin (T4) and triiodothyronine (T3)] regulate *in situ* metabolic

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processes, by making glucose available to the tissues, enhancing protein synthesis, increasing lipid metabolism, besides its cardiac and neural functions and in the productive performance of domestic livestock (TODINI et al., 2007; CUNNINGHAM, 2002; CAPEN and MARTIN, 1989). Thiourea- thio-uracil type goitrogen that interferes with the organic binding of iodine with tyrosine in the gland (MOSTAGHNI et al., 2005), consequently results in malfunctioning of the gland and exhibits antagonistic effects (hypothyroidism). There have been sporadic reports on hypothyrodism *vis-a-vis* productive and reproductive performances in ruminants, mainly cattle, available in literature (THRIFT et al., 1999). However, there seems to be little information on thiourea induced hypothyroidism, with special reference to its impact on liver and kidney functions in ovines. The present study was planned to assess the impact of thiourea induced hypothyroidism on liver and kidney function in Marwari sheep.

Materials and methods

Nine apparently healthy Marwari rams, aged 2 years and weighing between 35-40 (37.6 ± 0.6) kg were procured from the college sheep farm. The animals were maintained in conformity with scientific standards and were housed in clean, hygienic and well ventilated sheds. They were stall fed on a hay *ad libitum* and 200 g concentrate mixture was provided per day/animal, besides 4 hours of grazing on cultivated perennial pasture. The animals were monitored for their health status for a week prior to thiourea administration. During this period plasma levels of hormone, enzymes and blood metabolites were assessed. These data were used for the purpose of comparison with data generated after *per os* feeding of thiourea. Thiourea was given at the rate of 50 mg/kg b.m., *per os* to each animal for 7 days. Blood samples were collected with anticoagulant (heparin, 30 IU/mLblood) on day- 3, 5 and 7 of treatment by jugular vein puncture from individual animals. The plasma was separated, labeled and stored at -20 ºC in a deep freeze. On Days 3 and 5 of the experiment, thiourea was fed after blood collection. Hormone concentration, enzyme activities and blood metabolite concentrations in the plasma were estimated by standard methods. Thyroxine (T4) and TSH was estimated by the enzyme-immuno assay (EIA) technique using pathozyme T4 and TSH kit (Cat. No. OD377 and OD387) supplied by Omega Diagnostics (Bio-Rad Microplate reader 680). ALP (cat. No. 75MB100-40), AST (cat. No. 77MB101-50), ALT (cat. No. 76MB101-50), glucose (cat. No. 93DP100-74), blood urea nitrogen (cat. No. 81DP300-72), protein (cat. No. 83LS100-60), albumin (cat. No. 84LS100-60) and creatinine (cat. No. 85LS201-62) were estimated using diagnostic kits supplied by Span diagnostics by spectrophotometer (Systronics spectrophotometer 169). Statistical analysis of the data was carried out using analysis of variance test for unpaired data (SNEDECOR and COCHRAN, 1989).

Results

The clinical profile. Regular pre-feeding examination of the animals in the experiment for a period of 7 days, revealed clinical parameters (temperature, pulse, respiration, ruminal tonicity etc) within the normal range. This was suggestive of perfect health status prior to the start of the thiourea feeding trial. However, thereafter from day 4, the physical appearance of the animals progressively became weak, lethargic, and the animals developed moderate edema on the face and the lower parts of the hind limbs. There was a significant fall in daily feed intake (143 ± 2.3 g/day/animal). The animals tended to stretched their heads forward and hold them down and/or were seen pressing against the wall. The hair coat, although free from ectoparasites, looked dull, rough and matted at focal points on both lateral sides along the ventral mid-line and tail. At the end of experiment a decrease of 15 % in respiration rate, 10% in heart rate and a fall (26%) in body temperature was recorded.

The hormone profile. The plasma concentrations of thyroxine during the period of investigation are shown in Fig. 1a. The thyroxine levels fell to 23.82 ± 0.5 nmol/L during the period of feeding. The post thiourea feeding, progressive fall was 12.36% on day 3, 35.3% on day 5 and 53.3% on day 7. In comparison with pre-feeding plasma levels (51.01 ± 0.44 nmol/L), the fall was significant from day 3 onwards ($P<0.05$-0.01). The altered plasma concentrations of the Thyroid Stimulating Hormone (TSH) during the period of investigation are depicted in Fig. 1b. The TSH levels were observed to be elevated to 0.44 ± 0.04 micro IU/mL during the period of feeding. An initial fall of 10.51% was evidenced on day 3, and subsequently increased plasma concentrations of 18.28% on day 5 and 67.27% on day 7 were evidenced. The post feeding plasma concentration of TSH was significantly different from day 5 onwards ($P<0.05$- 0.01), in comparison with pre thiourea feeding values (0.268 ± 0.02 micro IU/mL).

The enzyme profile. The plasma concentrations of Alkaline Phosphatase (ALP) during the period of investigation are shown in Fig. 2a. The ALP activity increased up to 99.4 ± 4.51 U/L during the period. The progressive increases in ALP activity were respectively, 23.830% on day 3, 69.432% on day 5 and 49.982% on day 7. The values were significant from day 3 onwards ($P<0.05$-0.01) in comparison to pre-feeding values (66.27 ± 0.95 U/L). The plasma concentrations of Aspartate Amino Transferase (AST) during the period of investigation are shown in Fig. 2b. The AST levels increased to 79.69 ± 1.7 U/L. The progressive increase was 16.02% on day 3, 33.29% on day 5 and 70.75% on day 7. It was significant from day 3 onwards ($P<0.05$-0.01) in comparison to pre thiourea feeding values (46.67 ± 1.45 U/L). The plasma concentrations of Alanine Amino Transferase (ALT) during the period of investigation are shown in Fig. 2c. The ALT levels increased to 77.72 ± 2.64 U/L. The progressive increase was 78.99% on day 3, 113.01% on day 5 and 134.61% on day 7. In comparison to pre-feeding values (33.13 ± 1.19 U/L), it was significantly different from day 3 onwards ($P<0.05$-0.01).

The Hormone Profile

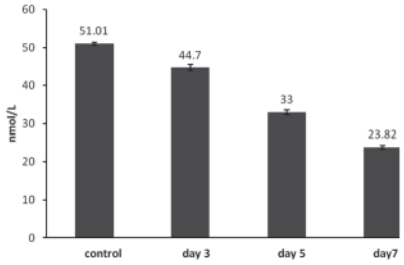

Fig. 1a. Altered levels of thyroxine (T_4)

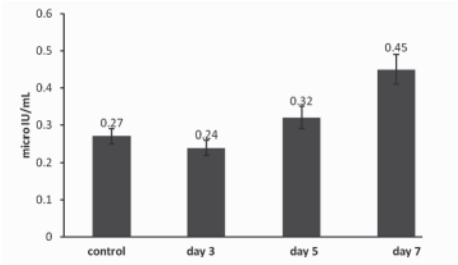

Fig. 1b. Altered levels of thyroid stimulating hormone (TSH)

The Enzyme Profile

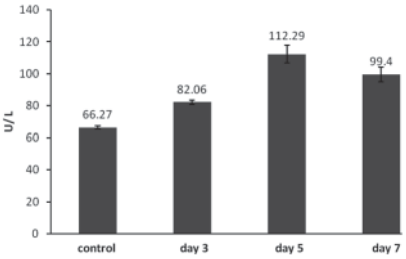

Fig. 2a. Altered activity of alkaline phosphatase (ALP)

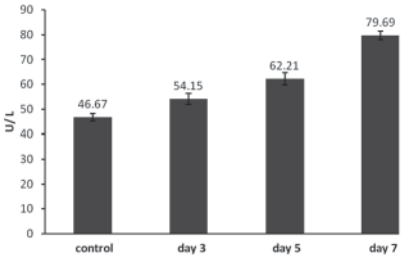

Fig. 2b. Altered activity of aspartate aminotransferase (AST)

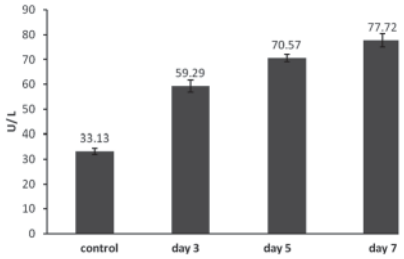

Fig. 2c. Altered activity of alanine aminotransferase (ALT)

The Metabolic Profile

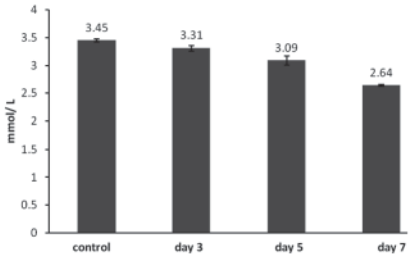

Fig. 3a. Altered glucose concentration

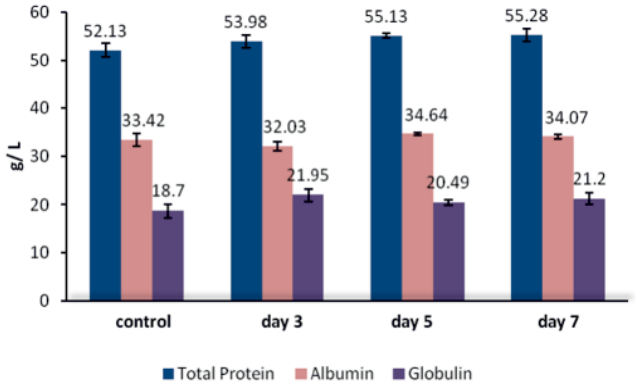

Fig. 3b. Altered protein concentration

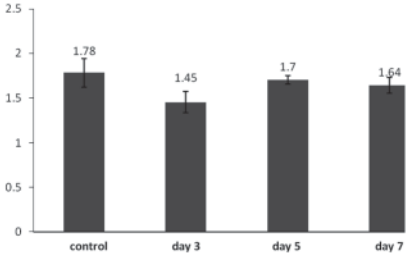

Fig. 3c. Altered albumin/ globulin (A/G) ratio

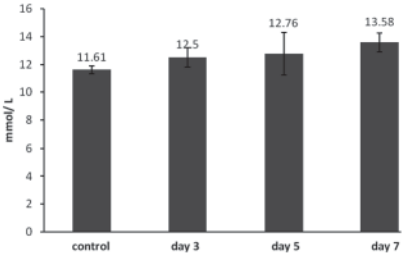

Fig. 3d. Altered blood urea nitrogen (BUN) concentration

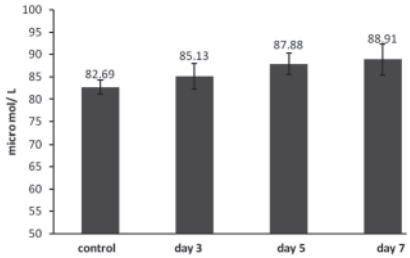

Fig. 3e. Altered creatinine concentration

The metabolite profile. The effect of thiourea feeding on the *in situ* metabolism parameters of the target animals was assessed, while synchronously monitoring altered levels of glucose, protein, blood urea nitrogen (BUN) and creatinine levels in circulation. The data so generated are depicted in Fig. 3. The plasma concentrations of glucose during the period of investigation are shown in Fig. 3a. The glucose concentrations in blood progressively fell to 2.64 ± 0.12 mmol/L. The fall was 4.09% on day 3, 10.33% on day 5 and 23.34% on day 7. The fall was significant from day 5 onwards ($P<0.05$-0.01) in comparison to pre thiourea feeding values (3.45 ± 0.03 mmol/L). The plasma protein profile during the period of investigation is shown in Fig. 3b. The total protein concentrations in blood progressively increased to 55.28 ± 1.27 g/L. The increase was 3.552% on day 3, 5.754% on day 5 and 6.035% on day 7. However, the altered levels were moderately significant ($P>0.05$). The albumin concentration increased to 34.07 ± 0.54 g/L. An initial fall by 4.170% was recorded on day 3; subsequently plasma concentration increased by 3.63% on day 5 and 1.95% on day 7. In comparison to pre-feeding values (33.42 ± 1.30 g/lit), the increase was not significant ($P>0.05$). The globulin concentration increased to 21.20 ± 1.12 g/L. The increase was 17.351% on day 3, 9.552% on day 5 and 13.337% on day 7. However, the post feeding increase was not significant ($P>0.05$) in comparison to pre-feeding values (18.70 ± 1.39 g/L). The A/G ratio progressively fell to 1.64 ± 0.09. The fall was 18.4% on day 3, 4.8% on day 5, and 8.1% on day 7. The post thiourea feeding fall was not significant ($P>0.05$). The plasma concentrations of BUN during the period of investigation are shown in fig. 3d. The BUN concentration progressively increased to 13.58 ± 0.67 mmol/L. The increase was 7.653% on day 3, 9.955% on day 5 and 17.014% on day 7. In comparison to pre-feeding values (11.61 ± 0.28 mmol/L), the increase was not significant ($P>0.05$). The plasma concentrations of creatinine during the period of investigation are shown in fig. 3e. The creatinine concentration increased to 88.91 ± 3.51 mmol/L. The increase was 2.947% on day 3, 6.265% on day 5 and 7.520% on day 7. The increase was not significant ($P>0.05$), in comparison to pre thiourea feeding values (82.69 ± 1.58 μmol/L).

Discussion

In this investigation the animals became lethargic and weak after feeding on thiourea. The heart rate, body temperature, respiration were depressed and feed intake decreased. The clinical profile of the animals reported here might be due to diminished levels of thyroid hormones in circulation that adversely affected the *in situ* functions of visceral organs. The consequential development of hypothyroidism directly reflects the altered metabolism at tissue level in the affected animals. Evidently, the animals suffered from lethargy and decreased appetite. Dryness, excessive shedding, and retarded growth of hair, are recognized as the earliest dermatologic changes to this effect (WILSON and FOSTER, 1992). The thyroid hormone stimulates the sodium pump at the cell membrane that accelerates oxygen consumption at tissue level (KANEKO et al., 1999). However, hypothyroidism dampens sodium pump activity, resulting in decreased oxygen consumption, depresses the metabolic activity of the affected animal and this ultimately results in reduced vital functions, such as respiration and heart rate etc. It adversely affects the *in situ* thermoregulatory mechanism ultimately resulting in a fall in normal body temperature, as witnessed here. The progressive decrease in circulating Thyroxine concentrations during the period of thiourea feeding trial is in conformity with earlier reports (NASSERI and PRASAD, 1987; MOSTAGHNI et al., 2005). Thiourea inhibits iodine oxidation or organic binding of iodine in the thyroid gland, thereby suppressing the biosynthesis of thyroxine (ADAMS, 2001). This leads to a significant decrease in thyroxine secretion from the thyroid gland. Thus, the decline in T4 is indicative of diminution of thyroid hormone synthesis and consequential hypothyroidism. Obviously thiourea feeding might have been reflected in decreased T4 concentrations in the blood and affected *in situ* metabolism during the post feeding period. The increase in the plasma levels of TSH as recorded in the present investigation is in concurrence with the earlier reports of GOMATHY et al. (2004) in hypothyroid dogs and COOPER (2001) in humans. The elevated levels of TSH might be due to the increased output of TSH from the pituitary gland as a compensatory mechanism in hypothyroidism (PANCIERA, 1994). TSH secretion from the pituitary gland is regulated by means of a negative feedback mechanism from the thyroid hormone secretion. The optimum functional capacity of the thyroid gland is adversely affected in primary hypothyroidism (CUNNINGHAM, 2002), as evidenced here.

The post thiourea feeding data on enzyme profile given above reflects the progressive development of lesions in the hepatocytes and hyperplasia of the bile ducts. A similar impact of thiourea feeding was documented earlier elsewhere (SAHA and MAITY, 2002; BARSHAM et al., 2005). Optimized hepatic function essentially requires normal levels of thyroid hormone in the circulation (HUANG and LIAW, 1995). Recently, MOSTAGHNI et al. (2008) reported pale, anemic, friable and enlarged livers in hypothyroid animals. The hepatocytes were swollen and exhibited fatty changes, especially at the periportal zone of

the hepatic lobule. They also observed bile duct hyperplasia, mononuclear cell infiltration and inclusion bodies in degenerated hepatic nuclei in hypothyroid animals. The elevated enzyme activity might be ascribed to depressed metabolic activity and/or increased extra cellular leakage from the injured hepatocytes. The disturbed cholesterol metabolism in the hypothyroid condition influences hepatic cell membrane permeability and the biliary excretory system, which is ultimately reflected in the elevated activity of the enzymes (SINGH et al., 2003). Thiourea feeding to animals might be toxic to hepatocytes/ inflicted trauma and may have initiated the onset of degenerative changes in hepatocytes. Post thiourea feeding resulted in a decrease in glucose concentration, in accordance with earlier reports (SINGH et al., 2003; MOSTAGHNI et al., 2005). The thyroid hormones are essential for *in situ* glucose metabolism (KANEKO et al., 1999). Decreased glucose levels are possibly due to altered gluconeogenesis, glycogenolysis, and absorption of glucose from the gastro intestinal tract, and decreased activity of hepatic glucose-6- phosphatase (RAHEJA and LINSCHEER, 1979) in hypothyroidism, as evidenced here. The moderate increase in protein concentration during post feeding of thiourea is in accordance with earlier reports (INGOLE et al., 2005; RAJGUDE et al., 2005). The hyperproteinemia might be due to a reduction in *in situ* catabolism of protein, resulting in a positive nitrogen balance. The increase in total protein (hyperproteinemia) may also be correlated with the increase in β-globulin levels in hypothyroidism, as a result of abnormal lipid metabolism (RAJGUDE et al., 2005). An increase in BUN and creatinine concentrations during post feeding of thiourea could be a sequel to the degenerative changes invoked by the thiourea. Recently, MOSTAGHNI et al., (2008) reports glomerular lipidosis and mild to moderate degrees of degenerative changes in the tubular epithelium in thiourea induced hypothyroidism. HASCHEK and ROUSSEAUX (1991) reports lipid plugging of renal glomeruli causing progressive renal failure in hypothyroid animals.

Conclusion

In conclusion, thiourea feeding induces hypothyroidism and generalized oxygen starvation at tissue level, and disturbs hepatic and renal functions. This leads to the altered hormone, enzyme and metabolic profiles discussed above. Uncontrolled and discriminate use of thiourea in crop fertilizer makes crop residue (used as animal fodder) rich in thiourea concentration. Feeding of such toxic crops to animals adversely affects growth, development, reproductive and productive performance of the animals and causes significant loss to the animal owner. This investigation has further paved the way for critically planned experimental studies on the soil-water-plant-animal relationship.

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SAŽETAK

Žetvene kulture sadrže ostatke tioureje, zbog njezine nerazborite uporabe kao gnojiva. Zbog toga je bilo vrijedno istražiti utjecaj dodataka tioureje na funkciju jetre i bubrega u odraslih ovaca. Hipotireoidizam je bio pokusno izazvan peroralnim davanjem tioureje u devet odraslih ovnova pasmine Marwari. Prije početka pokusa određene su kontrolne vrijednosti razina tiroksina (T4) u plazmi, tireostimulirajućeg hormona (TSH), alkalne fosfataze (ALP), aspartat-aminotransferaze (AST), alanin-aminotransferaze (ALT), glukoze, proteina, omjera A/G, ureje i nitrata u krvi (BUN) te kreatinina. Klinički nalaz u ovnova i prosudba navedenih pokazatelja razmatrani su 3. 5. i 7. dana nakon davanja tioureje. Na kraju pokusa ustanovljena je smanjena frekvencija disanja, smanjeni broj otkucaja srca, tjelesna temperatura i smanjeno uzimanje hrane. Značajan pad razine T4 i glukoze zapažen je 7. dana nakon davanja, dok su se vrijednosti za TSH, ALP, AST i ALT značajno povećale. Povećanje koncentracije proteina, BUN i kreatinina nije bilo signifikantno. Promjene u sadržaju hormona upućuju na hipofunkciju štitnjače. Povećana koncentracija enzima upućuje na hiperplaziju žučovoda, oštećenje hepatocita i žučovodne mreže. Nadalje, promjene u metabolizmu govore o smanjenom unosu energije i smanjenom rastu. Zaključno se može reći da davanje tioureje u hrani dovodi do hipotireoidizma, općeg nedostatka kisika u tkivima i poremećaja funkcije jetre i bubrega.

Ključne riječi: tioureja, hipotireoidizam, tiroksin, aspartat-aminotransferaza, alanin-aminotransferaza, alkalna fosfataza