Effect of induced Hypothyroidism on plasma cholesterol and bilirubin in Marwari Sheep

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Abstract

Hypothyroidism was induced in Marwari adult sheep (9 rams) by thiourea feeding (@ 50 mg/kg body weight). The blood samples were collected and analysed on 0 (control), 3rd, 5th and 7th day of thiourea feeding. The investigation was carried out to study the effects of induced hypothyroidism on plasma cholesterol and billirubin in Marwari sheep. Plasma cholesterol showed a non-significant (P > 0.05) effect of induced hypothyroidism however, it increased gradually with the subsequent phases of induced hypothyroidism. The analysis of variance (ANOVA) indicated a significant (P?0.01) increase in plasma bilirubin concentration during different phases of induced hypothyroidism in our study. Thus, Hypercholesterolemia and Hyperbilirubinemia are the salient clinical and biochemical features of induced hypothyroidism.

Keywords: Hypothyroidism, Sheep, Cholesterol, Plasma.

Introduction

Hypothyroidism is the commonest type of thyroid disorders encountered in the small ruminants. The clinical signs of hypothyroidism vary according to the diverse effects of thyroid hormones on cell metabolism (Sojka, 1995). Hypothyroidism may result in lowered resistance to infection, increased susceptibility to ketosis, decreased susceptibility to hypomagnesaemia and congenital goitre in the ruminants affecting both their production and reproduction.Thiourea (organic goitrogen) is one of most widely used fertilizers in modern agriculture. It can be used as a good substance to induce and study hypothyroidism experimentally.

The knowledge of blood metabolites in domestic animals is of great clinical importance in assessment of their health, nutritional status, diagnosis and prognosis of metabolic disorders as they are good indicators of physiological status of animals. Keeping in view, this study has been undertaken to observe the overall effect of induced hypothyroidism on the status of plasma metabolites i.e. cholesterol and bilirubin in Marwari sheep under agroclimatic conditions of Western Rajasthan.

Materials and Methods

Nine adult apparently healthy males (rams) of Marwari breed of sheep (above two years, weighing about 35-40 Kg) were included in our study. These animals were procured from the project scheme in the Department of Animal Nutrition, CVAS, Bikaner. These animals were provided with standard ration and water ad-libitum during the course of study. All experimental rams were kept isolated from the rest of the sheep. They were housed in clean and well ventilated sheds. The experimental plan was divided into following phases: Control (Phase-I) and Induction of experimental hypothyroidism

Control (Phase-I): Initially blood samples were collected from the normal animals, referred as control animals. The samples of these animals were estimated for plasma cholesterol and bilirubin. These parameters were treated as normal or control parameters.

Induction of experimental hypothyroidism: Each control animal was further subjected to thiourea feeding to observe the effects of induced hypothyroidism during the course of study on mentioned parameters.

To carry out the objectives of present study, thiourea was used as a choice of drug toinduce hypothyroidism @ 50 mg/kg bodyweight.

This stage was further subdivided into three phases.

3rd day of Thiourea feeding (Phase-II)
5th day of Thiourea feeding (Phase-III)
7th day of Thiourea feeding (Phase-IV)

Blood samples (10 ml each) were obtained with anticoagulant (heparin@ 30 IU/ml blood) by puncturing the jugular vein with least stress to animal under aseptic conditions directly into the hot air oven sterile tubes between 9.00 am to 9.30 am for control and 3rd,

5th and 7th days of thiourea feeding, respectively. Plasma for each sample was separated out on the same day in the laboratory. For separation of plasma the test tubes were centrifuged at 3000 rpm for 20 minutes and plasma was pipetted out in clear dry plasma tubes. Only non-haemolysed plasma samples were used. After collection, plasma was stored at -20°C in a deep freeze.

In our study, the effect of thiourea feeding was observed on the same animals which have been referred as control animals. On 3rd, 5th and 7th day of sampling during thiourea treatment, thiourea was given just after the sampling.

Estimation of plasma cholesterol and bilirubin

It was estimated spectrophotometerically by using spectrophotometer-169 of systronics

1. Cholesterol was estimated (mg%) by Wybenga and Pileggi method (1970) of kit as described in diagnostic reagent kit manufactured by Bio-lab diagnostic, Tarapur, Maharashtra, at 520 - 540 nm or green filter.
2. Bilirubin was estimated (mg%) by Malloy and Evelyn method cited by Varley (1988), at 540 nm or green filter.

Statistical analysis

The results were presented as Mean ± SE. The mean values were determined according to the effect of induced hypothyroidism. The data was subjected to analysis of variance (ANOVA) (Snedecor and Cochran, 1967). The critical difference among various means were worked out by "Duncun's New Multiple Range Test" (DNMRT) method.

Results and Discussion

The concentrations of plasma cholesterol and bilirubin have been illustrated in table 1 as mean ± SE (mmol/l and µmol/l, respectively).

In the present study, plasma cholesterol level was non-significantly (P>0.05) increased as a result of thiourea feeding and was directly propotional to the duration of thiourea feeding. This pattern is well in accordance with Nasseri and Prasad (1986 and 1987), Sokkar et al. (2000) and Mostaghni et al. (2005) in sheep. A similar finding has also reported by several workers in other species i.e. goats (Sreekumaran and Rajan, 1978 and Ramakrishnan et al., 1994) and broilers (Rajgude , 2005). Frank et al. (2003) also reported hypercholesterolemia in thyroidectomised (induced hypothyroidism) mares.

Hyperlipidemia and hypercholesterolemia might have resulted from increased mobilization of body fat reserves as a result of increased thyrotrophin (TSH) level induced by hypothyroidism. Low thyroid hormonal level in hypothyroid animals not only triggered enhanced thyrotrophin (TSH) secretion from pituitary, but also might have stimulated corticotrophin, and in turn, adrenal steroids, thereby increasing lipid mobilization through overlapping endocrine axis (McDonald, 1980). As thyroid hormones are involved in the hepatic catabolism of cholesterol and its biliary excretion.Therefore, the decreased rate of lipid metabolism with diminished intestinal excretion of cholesterol, decreased conversion of lipids into bile acids and other compounds and enhanced capacity for cholesterol transport in the blood in hypothyroidism also results in hypercholesterolemia (Jubb et al., 1985). Singh et al. (2002) reported elevated plasma lipid and cholesterol concentration in the goitrous goats. Treatment of affected goats with thyroxine resulted in lowering of plasma cholesterol level as compared to pre-treament value which supported the hypocholesterolemic action of thyroxine reported by Kaneko (1999). Similar findings were also reported by some authors in various species i.e. calves (Madej, 1972), canines (Gomathy et al., 2005) and human beings (Nordoy et al., 1976). Hypercholesterolemia and hyperlipidemia along with hypoglycaemia and poor body weight have been suggeseted as excellent indicators of suspected hypothyroidism in endemic areas of goitre (Nasseri and Prasad, 1987). Therefore, our investigation is helpful in clinical diagnosis of hypothyroidism.

The combined effects of decreased peripheral utilization to degradation and billiary excretion of fat can be attributed to the hypercholesterolemia during induced hypothyroidism in the present study. Thus, hypercholesterolemia can be considered as important clinical as well as biochemical characteristic feature in case of hypothyroidism.

The analysis of variance (ANOVA) indicated a significant (P>0.01) increase in plasma bilirubin concentration during different phases of induced hypothyroidism in our study . Mechanisms accounting for these changes in experimental hypothyroidism are a decreased biliary function, i.e., of enzyme activity, bile flow and output of bilirubin and salts (Van Steenbergen et al., 1989). As the liver has an important role in thyroid hormone metabolism and the normal circulating levels of thyroid hormones are also required for normal bilirubin metabolism (Fagiuoli and Ven Thiel, 1993 and Huang and Liaw, 1995). Our findings suggest a decreased excretion of bilirubin into the bile during induced hypothyroidism, resulting in transiently higher plasma levels, thus indicating hyperbili-rubinemia in response to induced hypothyroidism.

The earlier studies suggest that hypothyroidism is having effect on hepatic transport of endogenous bilirubin. Van Steenbergen et al. (1989) observed that bile flow and biliary output of bilirubin are reduced in severe thyroid failure. They studied the effects of thyroidectomy(induced hypothyroidism) and of thyroid hormone administration on the hepatic transport of endogenous bilirubin in the Wistar rat. They further reported that hypothyroidism resulted in an enhanced hepatic bilirubin UDP-glucuronosyltransferase activity and in a decreased p-nitrophenol transferase activity. It caused a cholestatic condition with a 50% decrease in

bile flow and bile salt excretion and an increased proportion of conjugated bilirubin in serum. The biliary output of unconjugated and monoconjugated bilirubins decreased in parallel by about 65%, whereas the excretion rate of the diconjugate dropped by only 47%, resulting in an increased di- to monoconjugate ratio in bile. Thus, supporting our findings. It is evident that thyroid function affects bile flow and composition, bilirubin excretion decreases in hypothyroid and increases in hyperthyroid rats (Gartner et al., 1972 and Layden et al., 1976).

Huang and Liaw (1995) reported the associations between thyroid and liver diseases of an auto-immune nature, such as between primary billary cirrhosis and hypothyroidism. They further revealed that thyroid diseases are frequently associated with liver injuries or biochemical test abnormalities. In addition, they suggested that antithyroid drug therapy may result in hepatitis, cholestasis or transient subclinical hepatotoxicity. It is concluded that hypothyroidism affects hepatic and biliary functions which may lead to hyperbilirubinemia due to above mentioned reasons.

There is paucity of information regarding the effects of induced hypothyroidism on blood/plasma bilirubin concentration, particularly in sheep. Hence, it was not possible to compare with other reports in this respect. It warrants further research.

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Table 1 : Mean ± SE concentrations of plasma cholesterol and bilirubin in the induced hypothyroidism in Marwari sheep.

Phases	Number of observations	CholesterolNS (mmol/l) Mean ± SE	% increase	Bilirubin** (µmol/l) Mean ± SE	% increase
Control (Phase-I)	9	1.803±0.062a	-	13.832±0.277a	-
		(1.648-2.128)	-	(12.825-15.219)	-
(Phase-II)	9	1.865±0.045a	3.408	21.280±1.187b	53.846
		(1.714-2.185)	-	(16.245-25.650)	-
(Phase-III)	9	1.885±0.091a	4.496	24.263±1.909b	75.412
		(1.305-2.346)	-	(16.074-30.780)	-
(Phase-IV)	9	1.976±0.081a	9.587	21.090±0.768b	52.472
		(1.500-2.381)	-	(16.758-25.650)	-

Note : Mean comparison have been made within different phases. Mean superscripted with different letters differ significantly (P< 0.05) from each other.

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