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Ameliorative effect of lignosulfonate on monocrotophos intoxicated lactating goats

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ABSTRACT

This study was carried out to investigate the ameliorative effect of lignosulfonate as a mechanical antidote on nutrient utilization and blood biochemical profile in cross bred (*Alpine* × *Beetal*) lactating goats. A total number of 20 cross bred goats were randomly assigned to four treatments with 5 goats in each. The dietary treatments consisted of the basal diet devoid of supplemental calcium lignosulfonate and monocrotophos (MCP) (control) or were supplemented with calcium lignosulfonate (2.5% DM basis), MCP (25 ppm) or both calcium lignosulfonate (2.5% DM basis) and MCP (25 ppm). At 0, 15, 30, 45 and 60 days intervals, blood samples were collected from jugular vein for the biochemical analysis. Goats were raised for 60 days and a metabolic trial for a period of 6 days was conducted after 45 days of experimental feeding to assess the nutrient digestibility, nitrogen balance and MCP residue in milk and urine. In present findings, intoxication with MCP did not affect ($P > 0.05$) digestibility, DM intake, body weight change, milk yield and nitrogen balance. However, blood acetylcholine esterase and transaminase activity significantly declined ($P < 0.05$) in MCP fed goats. Kidney function parameters (serum creatinine and urea concentration) were found similar ($P > 0.05$) in all treatment groups, indicating no adverse effect of treatments on kidney function. This study suggests that the supplementation of lignosulfonate as mechanical antidote in MCP intoxicated goats reduced its absorption and excretion through urine and milk while increasing its faecal excretion that may be beneficial in public point of view.

1. Introduction

Monocrotophos is being increasingly used to control a range of pests in agricultural crops in India (Agnihotri, 2000). Therefore crop residues constitute a significant part of feed and fodders of domestic animals are often contaminated with pesticides (Bhatnagar and Gupta, 1998; Raikwar and Nag, 2003; Nag and Raikwar, 2011). The feeding of these contaminated feed and fodders by the animals is main source of entry of pesticides into the animal body (Prasad and Chhabra, 2001; Mohan and Singh, 2013). After entering in blood, pesticide residues are distributed to different organs, tissues and excreted via urine, faeces and milk in lactating animals (Juliet et al., 1998; Cecchi et al., 2012; Huen et al., 2012). Organophosphates especially MCP is highly hazardous for human due to its contact, systemic and residual mode of action (Beynon et al., 1973; Kazemi et al., 2012b). Organophosphate pesticides (OPP) are very potent anti-acetylcholinestrase compounds and toxic xenobiotics that adversely affect the biological system (Malik et al., 1978a; Bayoumi et al., 1979; Singh, 2004; Kazemi et al., 2012a). Studies reported

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Table 1
Chemical composition (g/100 g, on DM basis) of experimental diet fed to lactating goats.

Attribute(s)	Concentrate mixture	Lucerne	Attribute(s)	Concentrate mixture	Lucerne
Dry matter	90.80	20.93	Nitrogen free extract	59.41	35.00
Organic matter	93.16	86.12	Neutral detergent fibre	28.00	41.70
Crude protein	20.70	15.34	Acid detergent fibre	26.00	33.40
Ether extract	3.49	2.86	Total ash	6.84	13.88

activated charcoal as mechanical antidote alleviates the toxicity of OPP in ruminants (Singh, 2004; Senthil et al., 2005). The use of commercial activated charcoal is difficult because of its high cost. Therefore, lignin as an alternate to activated charcoal was tried in present study due to its binding capacity to various pesticides under simulated gastro-intestinal conditions (Ta et al., 1999). Moreover, it is available cost effectively as lignosulfonate, a by-product of paper and pulp industry. Therefore present study was conducted to observe the ameliorative effect of lignosulfonate supplementation on nutrient utilization and blood biochemical profile in MCP intoxicated lactating goats.

2. Materials and methods

The experiment was conducted at National Dairy Research Institute (NDRI), Karnal, India. It is situated on an altitude of 250 m above mean sea level, latitude and longitude position being 29° 42″ N and 79° 54″ E, respectively. All procedures related to animal care were conducted with the approval of the Institutional Animal Ethics Committee (IAEC) constituted as per the article number 13 of the CPCSEA-rules, laid down by Government of India.

2.1. Animals, feeding and management

Twenty, 37.20 ± 0.1 kg body weight, 1–2 lactation cross bred (*Alpine* × *Beetal*) goats were selected from the herd of NDRI, Karnal, India. Deworming of all the animals was done before the start of the experiment. The goats were randomly assigned to four treatment groups ($n = 5$), on body weight and lactation basis. The dietary treatments consisted of the basal diet devoid of supplemental calcium lignosulfonate and MCP (control) or were supplemented with calcium lignosulfonate (2.5% DM basis), MCP (25 ppm) or both calcium lignosulfonate (2.5% DM basis) and MCP (25 ppm). Experimental feeding was continued for a period of 60 days. The nutrient requirements of goats were met by feeding concentrate mixture and lucerne fodder (*Medicago sativa*) (Ranjhan, 1998). Concentrate mixture consisting of maize 33%, deoiled rice bran 11%, mustard oil cake 12%, wheat bran 20%, groundnut cake 21%, mineral mixture 2% and common salt 1%. The chemical composition of the feeds offered to the animals viz. concentrates mixture and lucerne is presented in Table 1. A premix was prepared by mixing analytical grade MCP (Sigma Aldrich, USA) with maize grain flour. The premix and calcium lignosulfonate were mixed with concentrate just before feeding. Feed offered and residue left were weighed daily and analyzed for dry matter (DM), organic matter (OM), nitrogen (N) and CP (methods 942.05 and 984.13 of AOAC, 1995), NDF and ADF (Van Soest et al., 1991).

2.2. Blood sampling and biochemical analysis

Blood samples were collected in sterile vacutainer (Becton Drive, Franklin Lakes, NJ, USA) tubes from jugular vein puncture, posing minimum disturbance to the goats on 0, 15, 30, 45 and 60 days of dietary treatments before feeding (9.00 AM). Acetylcholinestrase activity (Pardio et al., 2001) and glucose (Hultmann, 1959) were determined in whole blood samples. Remaining blood samples were centrifuged at 1200 × *g* at 4 °C for 20 min to separate the serum for the analysis of creatinine, glutamate oxaloacetate transferase (GOT), and glutamate pyruvate transferase (GPT) activities at 37 °C reaction temperature using Span diagnostic kits (Span Diagnostic Ltd., India).

2.3. Metabolic trial

A metabolic trial for a period of 6 days was conducted after 45 days of dietary treatments for determination of digestibility and nutrient balance. All the goats were housed in individual metabolic stalls having facility of separate feeding. The diet was weighed daily and then offered to goat with 4 day adaptation period in the metabolic stalls followed by a 6 day collection period. Daily feeds offered and residue left was recorded to determine the net feed intake. The faeces of individual animals were collected from the floor while urine was collected in bags. Representative samples of feeds offered, residue left and faeces were collected daily and was taken in pre-weighed dried aluminium tray and dried at 100 ± 2 °C overnight for dry matter determination. After determination of dry matter, dried faecal sample for each goat was pooled for 6 days and used for chemical analysis. For N determination, representative faecal samples were kept in a pre weighed plastic container and known quantity of 1:4 H_2SO_4 solutions was added. Similarly, total urine voided was kept in separate bottle and known quantity of H_2SO_4 solution was added to prevent ammonia nitrogen loss.

2.4. MCP determination

Determination of MCP residues in feeds, faeces, urine and milk was done in three steps i.e. sample extraction, cleaning up and quantification using HPLC (WATERS, USA, C_{18} μ Bondapak column {300 mm × 39 mm, 10 μm particle size}) as per method described by Singh (2004). Acetonitrile was used for extraction of grounded samples of concentrate mixture, lucerne, faeces, milk and urine. For cleaning up of milk samples solid phase extraction cartridges (Discovery C_{18}, 3.0 ml, 500 mg, Supelco, USA) were conditioned over vacuum manifold assembly (Visciprep 12DL, Supelco, USA) thrice with methanol and thrice with acetonitrile. For cleaning up of concentrate mixture, lucerne, faeces and urine samples Dual Layer ENVI-Carb II/PSA solid phase extraction cartridge (500 mg/500 mg/6 ml Supelco, Sigma Aldrich, USA) were conditioned thrice with acetonitrile. After clean up, samples were evaporated to dryness under gentle stream of nitrogen and vacuum and reconstituted with 1 ml acetonitrile (HPLC grade). Finally, samples were analyzed using HPLC.

2.5. Statistical analysis

The data on nutrient intake, BW changes and digestibility of nutrients were statistically analyzed by using the general linear model of SPSS version 19.0 (IBM SPSS Statistic). The means in different treatment were tested for statistical significance using Tukey honestly significant difference test ($P \leq 0.05$). The blood biochemicals data were analyzed by repeated measure analysis with day of sampling as repeated measure and treatment group as between subject variable. The following statistical model was used:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha_b)_{ij} + \varepsilon_{k(ij)}$$

where μ is the overall mean; α_i effect of treatment i; β_j effect of period j; $(\alpha\beta)_{ij}$ interaction of ith treatment and jth period and $\varepsilon_{k(ij)}$ is as residuals error.

3. Results and discussion

3.1. Nutrient intake and body weight changes

The nutrient intake and body weight change of goats are summarized in Table 2. Nutrient intake in term of DM, CP, DCP, and TDN was not affected ($P < 0.05$) by supplemental lignosulfonate and MCP. Similarly daily milk yield was