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Assessment of nitrate content in green fodder samples of Salem District meant for livestock feeding

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Abstract

A study was conducted to estimate nitrate content in commonly used green fodder samples, Co-4, Co-3, Co-5 (Cumbu Napier Hybrid), Sorghum (*Sorghum vulgare*) and maize (*Zea mays*), collected from the fields of different villages of Salem (Tamil Nadu) and to evaluate the factors associated with nitrate accumulation in these green fodders. The nitrate was analyzed from collected green fodder samples by using score card method. The nitrate level analysis is estimated based on the reactions involving acetic acid and Bray's indicator as the coupling agents. The nitrate level was determined from collected 1322 green fodder samples showed that 11.80% samples had 0-100 ppm, 21.70% samples had 101-200 ppm, 29.87% samples had 201-500 ppm, 29.27% samples had 501-1000 ppm, 7.03% samples had 1001-2000 ppm and 0.30% samples had above 2000 ppm. From the result analysis, it has been suggested that green fodder samples above 2000 ppm of nitrate fodders has to be wilted before feeding to the dairy animals. Hence, laboratory testing of green fodder is always recommended, so that to prevent the nitrate toxicity incidence in dairy animals.

Keywords: Nitrate toxicity, green fodder, dairy animals, sorghum, acetic acid, forages

1. Introduction

Cow fed on high quality forage produce more milk with less supplemental concentrate than the cows fed lower quality forages. Forages with high concentrations of crude protein (CP) are considered high quality because feeding high protein forage cuts down the need of supplemental protein. Secondly CP content is positively correlated to energy content of forages. High protein forages generally are more digestible and provide more energy than low protein forages. (Kahn, 2005) [4].

Forages take up and assimilate nitrogen as NH_4^+ , NO_3^- and soluble organic compounds such as urea ($\text{CO}(\text{NH}_2)_2$) and amino-acids (Bose, 1996) [1]. Nitrate is the primary nutrient form of the nitrogen in soils and is a normal constituent of plants. Occasionally, excessive amounts of nitrate accumulate in plants and result in livestock mortalities. Outbreaks of nitrate toxicity due to consumption of fodder containing high amounts of nitrate have occurred in farm animals throughout the world. (Nicholls, 1980) [7]. Most commonly, nitrate poisoning occurs in cattle and sheep. In ruminants, nitrate is reduced by microbial reductases to nitrite. The rumen microbes utilize this nitrite by converting it into ammonia as a nitrogenous source. However, excessive nitrite gets accumulated in rumen, from where it is readily absorbed into blood stream and combines with ferrous ion of hemoglobin (Hb) to form met-hemoglobin (met-Hb). The met-Hb is a poor transporter of oxygen in the body and the animal suffers from oxygen deficiency (Kathirvelan *et al.*, 2019) [5,9].

In livestock, poisoning due to nitrate ions is influenced by several factors that include plant, environmental, management factors and health status of the animal. The plant factors are the most important amongst these because nitrate toxicity in livestock is chiefly caused by consumption of plants rich in nitrates. (Kathirvelan *et al.*, 2019) [5,9]. The factors that influence the accumulation of nitrate in fodder crops are species, stage of growth, part of plant, pH of soil, use of fertilizers and climatic conditions. These factors had not been studied recently and the guidelines found today in literature are based on limited research data obtained in the 60s and 70s, and have not been updated to more recent research and field experiences. Keeping this in view, factors contributing toward accumulation of nitrate in forages were studied and correlated with recent research and the field problems encountered in dairy animals due to excessive exposure to nitrate rich plants (Bose, 1996) [1].

2. Materials and Methods

All chemicals used were of analytical reagent grade, and doubly distilled water was used in the preparation of all solutions in the experiments. Hydrochloric acid solution (0.1N HCl) was prepared by 10 ml HCl in 1L Distilled water. Acetic acid (20%) was prepared by 20 mL Acetic acid in 80 ml distilled water. Bray's indicator prepared by 100g barium sulphate (BaSo₄), 10g manganese sulphate (MnSO₄.H₂O), 2g Zinc (metallic Zn), 75g citric acid, 4g sulphanic acid, 2g 1-naphthylamine are mixed. The bray's indicator is stored in blackened bottle away from light.

2.1 Sample Collection

The numbers of green fodder samples were collected based on the proportionate random sampling from the Salem district area. The Salem district area was around 5205.00 Km² (Thirunavukarasu, 2012) [8]. In Salem district, 1322 green fodder samples were collected from different villages of different blocks namely Attur, Ayothiappattinam, Gangavalli, Idappadi, Kadaiyampatty, Kolathur, Konganapuram, Magudanchavai, Mecheri, Nangavalli, Omalur, Panaimarathupatti, Pethanaickenpalayam, Salem, Sankagiri, Talavasal, Tharamangalam, Valapady, Veerapandi and Yercaud by using stratified random sampling/Multi stage random sampling. The source of green fodder samples commonly from Co-4, Maize, Co-3, Co-5, CoFS29 and Sorghum were collected for the estimation of Nitrate.

2.2 Nitrate determination

Nitrate content was estimated using Wiseman and Jacobson (1965) method. Cattle fed water Samples were taken in 1ml test tube and add 1ml of (20%) acetic acid and 0.5g of Brays indicator for the determination of nitrate. The pink color was observed and compared with score card value. The pink colour formation was due to the reduction of nitrate to nitrite by zinc and manganese sulphate. The reaction then followed by diazotization of sulfonic acid with nitrate ion and subsequently coupled with 1-naphthylamine to form pink colour (Kathirvel *et al.*, 2019) [5, 6].

3. Results and Discussion

3.1 Green fodder samples collection

Totally 1322 samples were collected from different villages. Among them, 317 (23.97%) from Co-4 samples, 308 (23.29%) from Co-3 samples, 24 (1.81%) from Co-5 samples, 168 (12.70%) from CoFS29 samples, 306 (23.14%) from sorghum samples and 199 (15.05%) maize samples. The

different green fodder samples collected were listed in Table 1 and figure-1. The amount of nitrate accumulated within the plant depends upon the rate of nitrogen uptake by the plant from the soil and the rate of its reduction by the plant. There is no accumulation when the rate of reduction equals the rate of uptake and when uptake exceeds the rate of reduction, nitrate starts getting accumulated. The nitrate accumulators and may cause sudden death in animals.

Table 1: Collection of green fodder samples

Total Green Fodder Samples	Co-3	Co-4	Co-5	Cofs29	Sorghum	Maize
1322 (100%)	317 (23.97%)	308 (23.29%)	024 (1.81%)	168 (12.70%)	306 (23.14%)	199 (15.05%)

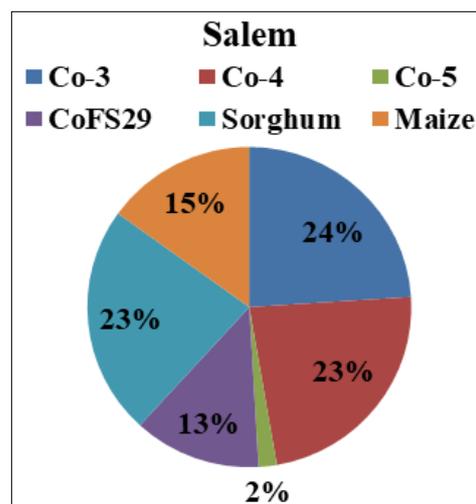


Fig 1: Collection of green fodder samples

3.2 Nitrate estimation of water samples

The nitrate content was estimated from different green fodder samples Table-2. The result showed that nitrate concentration found to be varying between samples. Among 1322 green fodder samples showed that nitrate content 11.80% samples had 0-100 ppm, 21.70% samples had 101-200 ppm, 29.87% samples had 201-500 ppm, 29.27% samples had 501-1000 ppm, 7.03% samples had 1001-2000 ppm and 0.30% samples had above 2000ppm. Nitrate can be detected in traceable amounts in all plants but it becomes dangerous when it exceeds the safe limit of 2000 ppm NO₃-N and forages having more than 2500 ppm NO₃-N are considered highly toxic.

Table 2: Nitrate estimation of Green fodder samples

Green fodder Samples	Nitrate Content (ppm)						Total
	0-100	101-200	201-500	501-1000	1001-2000	>2000	
Co-3	33 (10.41%)	53(16.71%)	101 (31.86%)	109 (34.38%)	21 (6.62%)	--	317 (23.97%)
Co-4	38 (12.33%)	112(36.36%)	96 (31.16%)	54 (17.53%)	07 (2.27%)	01 (0.32%)	308 (23.29%)
Co-5	--	06 (25.00%)	05 (20.83%)	10 (41.66)	03(12.50%)	--	024 (1.81%)
CoFS29	39 (23.21%)	48(28.57%)	53 (31.54%)	28 (16.66%)	--	--	168 (12.70%)
Sorghum	15(4.90%)	31 (10.13%)	86 (28.10%)	125 (40.84%)	47 (15.35%)	02 (0.65%)	306 (23.14%)
Maize	31(15.57%)	37(18.59%)	54 (27.13%)	61 (30.65%)	15 (7.53%)	01 (0.50%)	199 (15.05%)
Total	156 (11.80%)	287 (21.70%)	395(29.87%)	387 (29.27%)	93 (7.03%)	04 (0.30%)	1322 (100%)

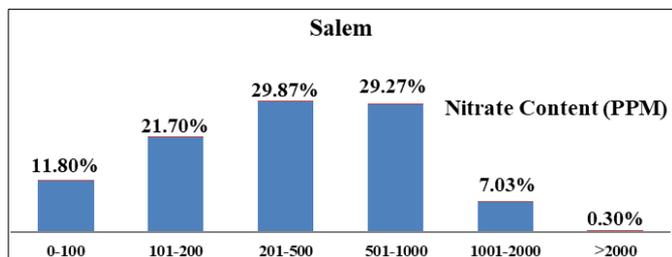


Fig 2: Nitrate estimation of green fodder samples

Nitrate accumulation varies with the stage of plant growth. Rate of uptake diminishes with the maturity of the plant. Therefore, immature (young) crop contain more nitrate than the mature crop. The concentration of nitrate differs with plants when accumulation occurs. To determine the distribution of nitrate in the plants, the nitrate content was determined in leaves, stem and whole plant of Co-4, maize, Co-3, Co-5, CoFS29 and sorghum [Table 2]. Results showed the variability in nitrate level in different plants. All forages exhibited similar results of having higher levels of nitrate in stem than in leaves. The difference of nitrate levels in stem and leaves was highly significant in Co-4, maize, Co-3, Co-5, CoFS29 and sorghum. This might be due to differences in the ability of roots to take up nitrogen from the soil. These findings have confirmed that plant parts vary in nitrate content; parts close to the ground contain more nitrates and as we go higher along the length of plant, nitrate content goes on decreasing. Roots and stems have more nitrate content, followed by leaves, whereas flowers and grains usually contain little or no nitrate.

Besides the above, weather conditions influence nitrate accumulation in plants significantly. Unfavourable weather conditions for plant growth, *viz.*, drought, frost, extreme cold and cloudy weather, may increase nitrate accumulation in plants (Carrigan, 1982). In the present study, nitrate level was significantly higher in forages when determined in adverse growing conditions, *viz.*, cloudy, cold with frost, wet in winters and very hot drought conditions in summer compared to nitrate concentrations found in same fields under normal weather conditions (Bose, 1996) [1]. In very hot summer, the nitrification of bacteria in soil increases many folds which leads to nitrate accumulation in fodder. The cold and cloudy weather decreases the nitrate reductase activity and hence increases nitrate accumulation by plants. The conditions detrimental for plant growth including frost enhance accumulation of nitrate by reducing the surface area of the plant available for evaporation and photosynthesis (Nicholls, 1980) [7].

Two outbreaks of nitrate poisoning in cattle due to Jumbo grass (sorghum hybrid) were reported in New Zealand. In both the incidents, the climatic factors were responsible for increasing nitrate content in forage. Accidental rain after a long dry summer, causing very rapid growth of grass, was a favourable condition for nitrate accumulation (Kahn, 2005) [4]. Similarly, sudden deaths due to nitrate poisoning occurred in cattle grazing ryegrass pasture for 6 hours in Australia. The quantitative analysis of ryegrass showed levels of 12.5% nitrate/nitrite on dry matter basis. Summer, newly sown pasture, regenerated lush grass and cloudy environment, contributed to high nitrate levels in the grazed pasture.

Soil type also plays an important role in nitrate accumulation. The plants grown in acidic and phosphorus deficient soils are known to have greater nitrate content. The uptake of NO_3^- is largely increased at slightly acid pH levels because of the

higher H^+ gradient across the plasma membrane at low pH, and possibly because increased H^+ influx reduces the membrane potential and facilitates NO_3^- uptake (Kahn, 2005) [4].

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