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Significance of dietary sulphur and sulphate reducing bacteria in lactating dairy cattle: A review

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Abstract
Sulphur is an important mineral for animal nutrition as it is integral component of many biologically active compounds like amino acid, vitamins, co-enzymes, cartilage, glutathione, fibrinogen, heparin etc. Biotin, thiamine and methionine are essential compounds in monogastric animal's diet but can be formed in rumen animal with the help of rumen bacteria and other can be manufactured with these. Generally sulphur contain approximately 0.15% of animal body weight but it may vary from species to species. Controlled amount of sulphur in dairy cattle can improve their performance in context of milk, digestion with the help of sulphate reducing bacteria, methane gas emission, but if given in dose more than the permissible level, may result into harmful effects.

Keywords: Dietary sulphur, dairy cattle, animal feed, sulphur oxidizing bacteria, sulphur reducing bacteria

Introduction
Sulphur (S) is an important mineral which have wide applications in the manufacturing of fertilizers, pigments, sulphuric acid, drugs, dyes, rubber, insecticides, explosives and detergents as well as inorganic salts and esters. Despite of being present uniformly in the nature, some countries consume more sulphur materials (Hall, 2018) [18]. Sulphur is also major nutrient for the growth and development of plants and animals.

Sulphur is an essential nutrient for animals because sulphur containing complexes, such as proteins, hormones, B-vitamins and many co-enzymes including CoA are requisite for their normal structural, metabolic and regulatory mechanisms (Goodrich and Garrett, 1986) [13]. Sulphur is also found in many other molecules like chondroitin sulphate, biotin, cartilage, glutathione, mucopolysaccharides, fibrinogen, lipoic acid, heparin, mucins and thiamine (NRC, 1989, 1998, 2006) [29]. In addition to these biologically active compounds, S is also found in sulphur-containing amino acids, such as cysteine, cystine, homocysteine, methionine and taurine. Almost all S-containing components present in the body except thiamine and biotin can be manufactured from methionine (NRC, 1996) [30]. So methionine, thiamine and biotin are considered as essential nutrients of monogastric animal's diet but in ruminant, microorganisms present in their stomach can produce these compounds from inorganic sulphate in the feed (Block *et al*., 1951) [4].

Sulphur comprises approximately 0.15% of animal body weight (NRC, 1989, 2006) [29]. According to National Research Council recommendation, the S concentration for the diets of beef cattle is 0.15% (w/w, on dry matter basis) but the maximum acceptable level of dietary S has been based on the percentage of only forage in the diet. Recommended daily intakes of S also vary from species to species for example for sheep, horses, beef cattle and dairy cattle, it is 0.14%-0.26%, 0.15%, 0.15%-0.2%, and 0.2%-0.25% respectively. This differences among species is due to differences in their metabolism such as cats can't synthesize taurine from methionine thus making it essential in their diets (NRC, 1985, 1988, 1989, 1996) [27].

Different sources of Sulphur
Most commonly used two diet sources for cattles are dried distillers grains with solubles (DDGS) and corn gluten feed, other corn products and other inorganic sources are ammonium sulphate, potassium sulphate, calcium sulphate, sodium sulphate and magnesium sulphate. These are by-products of ethanol industry, which increased recently due to growth of the

ethanol industry in North America. In the last few years, DDGS availability increases as cost competitive feed ingredient due to expansion of bioethanol industry in the United States and beyond. Only US had ~200 plants in operation producing ~35 million tonnes of corn based DDGS in 2013. Corn DDGS contain 11 to 17% ether extract and 30% crude protein (CP), of which 55% is ruminal indigestible, making it cost effective and an alternative interesting source of protein nutritional feedstuff for livestock (Getachew *et al*., 2004; Janicek *et al*., 2008; Penner *et al*., 2009; Schingoethe *et al*., 2009) [12, 17, 34, 35]. Therefore, DDGS may be used as a fat supplement to decrease enteric methane (CH_4) emissions from dairy cattles (Wu *et al*., 2015) [16].

However, DDGS are having more sulphur than corn (from 0.3% to more than 1% (Felix *et al*., 2011) [10] and this high content of Sin DDGS limits the DDGS level that can be added to diet. But when DDGS are included at low levels in feed, these can be beneficial for both beef (Felix *et al*., 2011) [10] and dairy cows (Kurokawa *et al*., 2013; Benchaar *et al*., 2013) [23, 2]. However, large amounts of DDGS included in the cattle diet can result into rumen acidosis, which further reduces dry matter intake (DMI) and feed digestibility because of sulphuric acid. Extra amount of DDGS in feed also increases sulphide production by rumen Sulphate Reducing Bacteria, increasing the threat of occurrence of sulphide-associated polioencephalomalacia (PEM) (Felix *et al*., 2011) [10]. That's why, addition of DDGS is limited to 40% of dry matter (DM) in final diets of cattles.

The effects of different sources of sulphur *viz*. Na_2S, $Na_2S_2O_4$, Na_2SO_3, and Na_2SO_4 for nutrient values and having different sulphur concentrations (0.346, 0.692 and 1.038%) on the characteristics of fermentation of corn DDGS in vitro culture were investigated by He *et al*., 2017 [24] in two different in-vitro experiments. Depending upon on the chemical composition analysis and sampling and analysis, a great variation in the amount of S and proximate nutrients of DDGS was found. In 1st Experiment, S-source showed a significant effect on the gas production parameters from Na_2SO_4, while Na_2S produced more gas at faster rate within 48 h as well as higher digestibilities with more energy supplies than sulphur from Na_2SO_3 and $Na_2S_2O_4$. Also other parameters like ammonia-nitrogen (NH_3-N), volatile fatty acids (VFA) profile were not affected by sulphur source. In 2nd Experiment, there was no significant effect on the fermentation characteristics of DDGS with increasing S content. So a regular chemical analyses are required to make full use of DDGS and the valence state of Sin DDGS exerts its effect on *in vitro* fermentation features. Also no dose-related effect of S on the fermentation in a short-term in-vitro culture was observed. Hence the survey showed that there was a huge difference in the concentrations of S and proximate nutrients of DDGS used for feeding; consequently resulting in variable gas production and energy supplies; though, the S level showed no effect on the fermentation characteristics in in-vitro rumen culture.

In other experiment done by Bouchard and Conrad (1972) [6], the effects of S supplementation of rations for lactating cows were measured. Two experiment were conducted simultaneously, in first experiment, a basal diet (R 1) 0.10% S as semipurified mix and corn silage were given in a 1:1 ratio on a dry basis. Sodium sulfate was also added to the (R 2) to produce rations containing 0.15% and 0.18% S (R 3). The S content was also increased upto 0.18% by supplementation with methionine hydroxy analog (R 4). Sulphur supplementation of diet increased dry matter intake and digestibility. Methionine hydroxy analog fed at 43 g per day decreased dry matter intake to the intake of the S lacking diet without disturbing daily milk production. Both sodium sulfate and methionine hydroxy analog improved the sulphur balance of lactating cows with supplements of 0.15 and 0.18% S in the whole feed. In Exp. 2, a basal diet containing ~0.06% sulphur was supplemented with either sodium sulfate or a mixture of magnesium sulfate S and potassium to 0.18 and 0.24% in the complete diet. While low- S containing diet caused the sulphur balance to be negative. It was found that dietary 0.12% sulphur would approximate sulphur balance and ~0.18 sulphur allow for a mean positive balance of 4 g of sulphur daily in cows producing 8-37 kg milk.

Sulphur amino acids are an essential but small component of some proteins. Ratio of S: N in animal products varies from 0.055 to 0.068, so requirements of N and S are closely correlated. In addition, S has long been recognized as essential to ruminal microbes. Ruminant microbes provide ruminants with the amino acids cysteine, cystine and methionine, and the vitamins thiamine and biotin (Kazemi-Bonchenari *et al*., 2014) [21]. Sulphur or the amino acids or vitamins must be provided in the diet. As noted above, improving environmental conditions may actually raise a need for more close monitoring of sulphur and other trace minerals in forages. This is particularly important for animals receiving most of their nutrition from grazing. Ruminant diets should contain 1.0 to 3.0 g S kg^{-1} of dry matter (DM). Critical level of S required in diets of ruminants to avoid depressing effects on forage intake and digestibility is 1.8 g S kg^{-1} DM (Min). Concentrations above 3.5 g S kg^{-1} DM in diet have been associated with decreased 5 intake and consequently milk production. S plays an important role in the in the DCAD of up-close dairy cows and has been used extensively to control DCAD (Cherney and Cherney, 2015) [19].

Sulphur metabolising microorganisms

Microorganisms with capability of oxidizing reduced sulphur compounds with sulphate as an end product are known as sulphur oxidizing microorganisms (SOM), while another group having ability to convert sulphate into reduced form is known as sulphate (SO_4^{2-}) reducing. Microorganisms play a significant role in S transformations. Sulphate is taken up as a nutrient and reduced to sulphide, which is then incorporated into sulphur-containing amino acids and enzymes (Friedrich *et al*., 2001) [11]. Sulphur undergoes so many biological conversions in the nature, which are carried out absolutely by microorganisms. Most of the S is absorbed by plant roots in the form of sulphate (SO_4^{-2}), which further undergoes a series of conversions before its incorporation into the original S requiring compounds (Katyal *et al*., 1997) [20]. The transformations of inorganic sulphur compounds in nature have been formalized in the so-called sulphur cycle (Chaudhary, 2018) [7].

Most of the dietary S swallowed by ruminant cattles is transformed into sulphide by rumen microorganisms, primarily by rumen bacteria, with S-containing amino acids being fermented to sulphide and sulphate reduced to sulphide by ruminal sulphate reducing bacteria (SRB) (Coleman, 1960). Although the bacteria in the rumen are present in small amount, but sulphate reducing bacteria may have significant influence on feed diets of cattle with high S content, such as (DDGS) dried distiller grain with soluble (Wu *et al*., 2015) [16].

Depending on soil and growing conditions, straw is also deficient in the nutrients required by the microbes that digest straw e.g., Co and S along with the minerals essential to the well-being of the animal (P, Na, Mg, Cu and Zn).

Metabolism of sulphur in rumen

S-containing amino acids and sulphate are metabolized in order to produce biologically active S-compounds. Absorbed sulphide is competently metabolized in the liver to SO_4^{2-}, with high first clearance (NRC, 2006) [32]. S-containing compounds are removed by both routes-renal and biliary routes. The quantities of sulphur elimination from renal and biliary ways can vary depending on the ingested feed form. The greatest percent removal of sulphate was found via urine in sheep (Bird, 1972) [3]. The absorbed sulphide well undergoes hepatic metabolism to sulphate, which is removed by the urine (Hall, 2018) [18].

Rumen microorganisms convert a percentage of dietary S-compounds to sulphide, which further incorporated into microbial S-containing amino acids, biotin, thiamine and other microbial S-metabolites or absorbed as sulphide. In addition to gastrointestinal absorption of sulphides, hydrogen sulphide (H_2S) can be absorbed through respiratory epithelium. Large amounts of sulphide, as H_2S formed in the rumen can be removed, inhaled and absorbed (Dougherty *et al*., 1965) [9].

One recent study has involved biologically active forms of thiamine *viz*. low brain concentrations of thiamine pyrophosphate that is important in many metabolic pathways (Amat *et al*., 2013) [1]. A minor drop in the blood thiamine can also be seen in certain animals, where thiamine supplementation with high sulphate/sulphur-associated PEM suppresses the clinical disease (Olkowski *et al*., 1992) [33]. So indicate that the sulphide or some S-metabolite is either competitively inhibiting the cellular uptake/utilization of thiamine, or its therapeutic doses reduce the effects of sulphide on cytochrome C Oxidase (Hall, 2018) [18].

Sulphur utilization and excretion

In an experiment conducted by Morris *et al*., 2018 [8], it was found that compared with a control diet (CON), feeding cows the supplemental fat and phosphorus (DG) diet increased the S intake by 93% and fecal and urinary excretion by 17 and 265%, respectively. Urinary sulphur excretion to S intake ratio also increased for cows fed DG compared with CON. Total-tract sulphur digestibility also increased for cows fed the DG compared with CON feed. Further, total plasma sulphur and sulfate increased for DG versus CON, while addition of monensin to the DG diet did not affect sulphur utilization and excretion. Even though sulphur consumption increased both fecal and urinary sulphur elimination, the increase in urinary sulphur excretion was significantly greater than fecal sulphur excretion. Total plasma total sulphur can be affected by many factors like sulphur intake, its absorption in the rumen (as inorganic sulphur) and small intestine (microbial sulphur) and inhaling hydrogen sulphide produced from the rumen.

Benefits of dietary sulphur on Milk production and other parameters

Sulphur is a significant element in maintaining the pH balance of the blood of animals.

After feeding dairy cattle with hydroxyl methionine (S containing amino acid), there was stimulatory effect on milk production and also a positive result was obtained in preventing bovine ketosis (Griel *et al*., 1968) [15].

Increased S content resulted in decreased methane (CH_4) emission but unaffecting the total archeal population. Also the population of SRB was amplified in a sulphur dependent manner suggesting that dietary sulphur and buffering capacity affect rumen fermentation and sulphide production (Wu *et al*., 2015) [16].

Ruminal volatile fatty acids and ammonia-N at 5 h after feeding of 20% DDGS were decreased compared to Control, whereas protozoan count at 2 h after feeding of 20% DDGS was higher than that of 10% DDGS. Milk yield of cows fed DDGS diets was greater than that of Control. It was found that DDGS feeding improved milk yield, as well as CLA synthesis under a high dietary neutral detergent fiber (NDF, 45.9-46.6%) condition (Kurokawa *et al*., 2013) [23].

In other study, milk production increased with increasing DDGS proportions in the diet. After feeding the 30% DDGS to cows, 4 kg/d more milk was produced than with 0% DDGS supplementation. The higher milk may be due to increased protein yield due to greater supply of AA due to (1) an increased feed N intake (or) (2) a reduction in protein degradation in rumen, branched-chain VFA molar proportions, protozoa numbers, and because of soybean meal (Benchaar *et al*., 2013) [2].

Effect of deficiency of Sulphur in animals

A shortage of sulphur in the animal feed may have detrimental effects on their performance. Minimal deficiency of sulphur may be noticed with many symptoms like decreased microbial synthesis, supressed fibre digestion due to slow growth of microorganisms in cattle rumen, slow growth process, decreased feed intakes, reduced milk production, while severe deficiencies may result into weight loss, dullness and slow movement, reluctance to eat, excessive salivation and even death in worst cases (Hall, 2018; Wu *et al*., 2015) [18, 16].

Drawbacks or limitation of sulphur

If DDGS is given to cattle at higher rate, it may result into high buffering capacity and possibly alkalinity can be increased causing the adverse effects associated with feeding DDGS (Wu *et al*., 2015) [16].

Ruminants are more delicate to the lethal effects of dietary sulfate/sulphur due to microbial conversion to bioactive sulphur species in the rumen. Therefore both dietary and water sources of sulphur must be factored into the total daily intake in order to monitor potential risk. Dietary feed sources having high sulphur concentrations are frequently overlooked are DDGS and gluten feeds. Apart than dietary sulphur, other S-containing compounds can also be toxic. Sulphur dioxide (SO_2) gas from industrial, H_2S sulphide gas from manure, natural gas and crude oil production may be lethal to livestock (Hall, 2018) [18].

Sulphur toxicity may be categorised into three main classes

1. First one is acute oral poisoning
2. Second is direct sub-acute to chronic toxicosis
3. Third is indirect subacute to chronic toxicosis. (Hall, 2018) [18]

The indirect subacute to chronic effects of too much S are seen in ruminants, due to conversion of S-compounds to sulphide, which further can form insoluble salts with zinc and copper (Suttle, 1974) [36], may also react with molybdenum forming thiomolybdate complexes, which efficiently bind copper making it non available (Suttle, 1991) [37]. Reduced serum and wool selenium (Se) have been found due to increased dietary sulphate (White and Somers, 1977; White, 1980) [40, 39]. Increased soil sulphate also inhibits uptake of selenium by plants, thus increasing the probable risk for inducing a selenium deficiency in consuming herbivores (Newman and Schreiber, 1985) [26], which is an important mechanism in these grazing animals (Hall, 2018) [18].

Increased amount of dietary sulphur have lethal effects on performance of cattle and carcass features (Bolsen *et al*., 1973; Loneragan *et al*., 1997) [5, 25]. Kung *et al*. (1998) [22] reported that augmented dietary SO_4^{2-} may result in respiratory disorders and polioencephalomalacia (PEM). Within the rumen, sulphate is reduced to sulphide by SRB and the sulphides then bind in the rumen to form hydrogen sulphide H_2S. Hydrogen sulfide may be aspirated into the lungs from the rumen and in excess can cause PEM (Gould, 1998) [14]. For example, out of 40 steers fed with 50% DDGS in combination with DRC, 6 died or exhibited symptoms associated with PEM (Uwituze *et al*., 2011) [38].

Conclusion

Dietary sulphur is an important component of cattle feed stuff and may be result in beneficial effects such as proper growth, digestion and intake of dry matter, increased milk amount and protein content, reduced methane emission, if taken up to admissible level. Also deficiency of sulphur causes many deformities in animals including death in severe cases but sulphur supplementation may have harmful or lethal effects if dose increases from permissible level i.e. 0.15% of body weight as given by NRC. Therefore diet and water sulphur content should be monitored properly before feeding to cattle.

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