Effect of Dietary Urea and Sulphur in the Immune Response of Sheep Vaccinated Against Caseous Lymphadenitis

Khaled B. Alharbi

Department of Veterinary Medicine, College of Agriculture and Veterinary Medicine, Qassim University, P. O. Box 6622 Buraydah 51452, Qassim, Saudi Arabia
kbhrby@qu.edu.sa

Abstract: Abstract. This experiment has been carried out to study the effect of urea, sulphur or urea plus sulphur on the immune response of sheep vaccinated with a caseous lymphadenitis (CLA) bactrin. The urea, sulphur or urea plus sulphur were incorporated at the rate of 1g/kg (0.1 %) in a concentrate feed ration made of barley, wheat bran and salt. Twenty Najdi ewes were ear-tagged and divided into 4 groups of 5 animals each. Group 1 ewes were fed on the concentrate ration containing 0.1% urea. Group 2 ewes were fed on the concentrate ration containing 0.1% urea plus 0.1% sulphur. Group 3 ewes were fed on the concentrate ration containing 0.1% sulphur. Group 4 ewes were left as controls and fed on the feed ration only. All ewes had free access to alfa alfa (Medicago sativa) and drinking water. After one month, the ewes in the four groups were vaccinated with the CLA vaccine. A booster dose of the vaccine was given after 2 weeks from the initial vaccination. The ewes were then mixed with 5 heavily infected rams with abscesses to act as source of infection and to breed. The ewes were observed for abscess development pre- (during the feeding period) and post vaccination for 10 months. Ewes were bled before and at 2 weeks after vaccination and at 2 weeks after booster dosing, for the determination of serum Hb, PCV, AST activity, creatinine concentration and the titre of antibodies in the various groups. One ewe from group 2 (fed on urea + sulphur) and two ewes in group 1 (fed on urea) developed abscesses on the head after 3 weeks from the start of the experiment (before vaccination). The vaccine raised the level of immunoglobulins in serum of all groups and kept all animals free of abscesses for a period of seven months. The blood and serum constituents analyses results showed that urea, urea plus sulphur or sulphur incorporated in the feed with the specified concentrations were safe as indicated by the non-significant changes. However, it was observed that 2 ewes: one from group 2 and one from group 3 gave birth to paralyzed lambs. When these were post-mortemed, both had brain malacia. This is possibly due to sulphur effect. In conclusion, feeding concentrate rations containing 1 gm/kg sulphur, urea or sulphur plus urea did not affect the general health of experimental animals. It also did not affect positively or negatively the immune response of the ewes to the administered CLA vaccine.
[Khaled B. Alharbi. **Effect of Dietary Urea and Sulphur in the Immune Response of Sheep Vaccinated Against Caseous Lymphadenitis**. *Life Sci J* 2014;11(5):52-58] (ISSN:1097-8135). http://www.lifesciencesite.com. 7

Keywords: Urea, sulphur, caseous lymphadenitis, brain malacia.

1. Introduction

The dual effect of vaccination and oral feeding of urea and sulphur on the immunity of sheep against caseous lymphadenitis are less documented in the literature. Sulphur is necessary for the formation of the essentially sulphur containing amino acids by the ruminal bacteria. Grimble and Grimble (1998) reported that pro-inflammatory cytokines mediate widespread changes in protein metabolism and that amino acids released from peripheral tissues fulfill a number of functions such as acting as substrate for acute phase protein and immunoglobulin synthesis and, together with polyamines, in the replication of immune cells. Demands for specific amino acids may outstrip the supply from endogenous sources and it has been suggested that sulphur amino acids, and amino acids that are metabolically related to them, may be required in increased amounts in immunological reactions (Grimble and Grimble 1998). Protein deficiency impairs the acute phase response in inflammation. However, sulfur amino acids insufficiency compromise glutathione synthesis, to a greater extent than hepatic protein synthesis, in the presence and absence of an inflammatory stimulus. The resulting effect may be compromised antioxidant defenses. Function of T cells is dependent on intracellular glutathione concentrations and may also be affected by sulphur amino acid insufficiency. It has been suggested that the increased N excretion, which occurs during the immune response, is a reflection of a relative imbalance in the profile of amino acids released from peripheral tissues and the requirements imposed by the synthesis of substances involved in the acute phase response.

Macrophage dysfunction due to high blood urea appears to be a major site of impairment of host defenses. Hibbs et al. (1988) showed that nitric oxide (NO) may be a mediator of macrophage-mediated cytotoxicity. More recently, Vallance et al. (1992)

described and discussed the inhibition of NO synthesis due to retained endogenous factors in uremia. It is therefore conceivable that the dysfunction of macrophages in uremia may indeed in many physiological roles have been ascribed to NO in various tissues and organ systems, such as control of systemic blood pressure, platelet aggregation, control of renal hemodynamics, regulation of renal tubular transport, immune defenses, and neuronal signaling in central and peripheral nervous systems (Star, 1993). In the area of immune defense mechanisms, NO, which is released by macrophages on induction with bacterial endotoxins, g-interferon, or tumor necrosis factor, is believed to function as the effector molecule mediating the cytotoxicity. The NO inhibits the iron-containing enzymes in the target cells, including several enzymes involved in mitochondrial respiration (Iyengar et al., 1987). In addition, NO also inhibits DNA synthesis in the target cells. Thus NO may mediate the macrophage-dependent killing of bacteria, protozoa, and fungi. Thus, although the increased susceptibility to infections that occurs in uremic syndrome has been largely attributed to lymphocytopenia and leukocyte dysfunction, impairment of NO-mediated macrophage cytotoxicity may play an important role in the immune dysfunction in uremia.

The role of sulphur in the availability of copper to sheep is very well documented in the literature (Underwood, 1981). High concentrations of sulphur together with molybdenum in the diet will substantially reduce the biologically available copper for sheep resulting in all those symptoms of copper deficiency, including immune dysfunction and central nervous system (CNS) pathology.

Field studies have shown that cattle fed high S and low Cu show low blood B1 levels and succumb to PEM (brain malacia). Copper, Mo, S and B1 interactions and the associated disease syndromes that occur due to imbalances of these, namely, Cu deficiency, Cu toxicity and PEM "enzootic ataxia" in newborn and "swayback" in lambs occur due to a loss of cytochrome oxidase enzyme activity, bone fragility and cardiovascular disorders due to a deficiency of lysyl oxidase and therefore a lack of elastin - collagen cross linkages, anaemia due to a derangement in mobilization and transport of Fe as a consequence of low ferroxidase activity of the ceruloplasmin enzyme, coat colour changes in cattle and sheep and a loss of crimp ("steely wool") in sheep due to a lack of disulphide linkages in keratin, low immuno-competence due to low superoxide dismutase enzyme activity, swelling of joints, increased brittleness of bones, and loss of compact bone from the shaft of long bones due to a deficiency of the enzyme lysyl oxidase, an important Cu-containing enzyme involved in cross-linking of connective tissue, and loss of hair colour due to a deficiency of tyrosinase

The inflammatory reaction is also affected. For example, selenium, copper, zinc, sulphur and manganese are essential to the functioning of antioxidant enzymes and protect against oxidative damage to cells, resulting in decreased inflammation (McClure, 2003; Lee et al., 2002; Keen et al., 2004; Kidd, 2005; Park et al., 2004). A second general mechanism is in maintaining the processes and fidelity of DNA replication and transcription and RNA translation, as is the case for magnesium, sulphur and zinc (Guthrie, 1986; Lee et al., 2002; Keen et al., 2004; Grimble, 2001). A third mechanism, active for zinc and selenium, is via their role in regulating the transcription of relevant genes, including those encoding pro-inflammatory products, cytokines, hormones, neuroendocrine agents and receptors (Keen et al., 2004; McKenzie et al., 2001; Prasad, 2001).

Excess sulphur in the feed will affect the availability of other minerals that can adversely affect the immunity of the ruminant animal such as copper, selenium and zinc (Underwood, 1981). Copper or selenium deficiencies might cause health as well as similar paralytic clinical signs induced by sulphur excess (Jones et al., 1997). The aim of this study was to investigate some of the possible factors that affect the outcome of vaccination against CLA.

2. Material and Methods

Experimental animals and protocol of work: Twenty ewes and 5 Najdi rams were bought from Buraydah animal market. The ewes were ear-tagged and divided into 4 groups of 5 animals each. Group 1 ewes were fed on a concentrate ration containing barley, wheat bran and salt plus urea at a concentration of 0.1%. Group 2 ewes were fed on the same concentrate ration and urea and sulphur at a concentration of 0.1% each. Group 3 ewes were fed on a concentrate ration plus sulphur at a concentration of 0.1%. Group 4 ewes were left as controls and fed on the same concentrate ration. All animals had free access to berseem (alfa alfa) and drinking water. After one month, the ewes in all groups were vaccinated with a killed formalized vaccine prepared from a local strain of Corynebacterium pseudotuberculosis, the cause of caseous lymphadenitis (CLA). A booster dose of the vaccine was given after 2 weeks from the initial vaccination. Then all the animals were mixed with 5 rams heavily infected with CLA to act as source of infection and to breed. (A general protocol for the experiment is shown in Table1.

All the ewes were observed for development of CLA abscesses during the feeding period and after vaccination for 10 months to record the effect of the feed and the protective effect of vaccination. The ewes were bled three times: before and 2 weeks after vaccination and at 2 weeks post booster vaccine injection. Two samples of blood were collected each time. One sample was collected in EDTA-tubes for hematological studies and the other sample was collected in plain tubes and allowed to clot overnight to obtain serum. Serum was used for the determination of serum constituents and for immunological studies. The EDTA-blood was used for the determination of packed cell volume (PCV) and haemoglobin concentration (Hb). The (PCV) was determined by the micro-haematochrit centrifuge. The concentration of haemoglobin was determined by an automated auto-analyzer.

The activity of the enzyme AST and the concentrations of albumen, globulin, creatinine in serum were determined by commercial kit sets using a spectrophotometer.

Enzyme linked immunosorbent assay (ELISA):

ELISA was standardized using antigen of local C. pseudotuberculosis isolates to detect the subclinical infection as well as to evaluate the vaccine potency. Standardization was done as follows: Test antigen was prepared from a PLD-positive C. pseudotuberculosis isolate. The bacterial isolate was grown in brain heart infusion broth at 37ºC for 48 hours. The bacterial cells were spun down at 3000 RPM for 10 minutes. The bacterial pellet was washed 3 times in phosphate buffered saline (PBS) and suspended in 5 ml of PBS after the last wash. The bacterial suspension was sonicated for 30 seconds.

ELISA test:

Serum samples of vaccinated animals as well as animals of flocks with history of abscesses were tested. ELISA was carried out using C. *pseudotuberculosis* antigen at the proper dilution as indicated by the results of the checker-board titration. Wells of 50 ELISA plates were coated with 50 microlitres of the antigen at the proper dilution (in carbonate/bicarbonate buffer). The coated plates were kept at 4ºC for an overnight, then washed 3 times with PBS/T, dried and kept at 4ºC in tight plastic bags. When needed, an ELISA plate was taken out of the refrigerator and 50 microlitres of PBS/T were distributed into each well. Twenty five microlitres of each one of the test serum samples, of different animals, were delivered into a corresponding well of row A. (A1-A12). The samples were 2-fold serially diluted in the direction A-H and the plate was kept at room temperature for 30 minutes. The test steps were completed following the same steps of the antigen titration from blocking to reading and interpretation. Negative and positive control samples were tested in parallel with the test samples.

3. Results

Development of Abscesses:

A - Development of CLA abscesses before vaccination (during feeding with urea and sulphur):

Two ewes from group 1 (fed on urea) and one ewe from group 2 (fed on urea + sulphur) developed abscesses on the head three weeks after the start of feeding experiment (Fig. 1). The ewes of the group 3 (fed on sulphur) and group 4 (control group) remained free of the disease.

B – Development of abscesses after vaccination:

Abscess appeared in one sheep from each of the four groups at eight months post-vaccination. (This means that the vaccine was protective for a period of seven months post-vaccination and that its efficacy was not affected by the urea, sulphur or both incorporated in the feed (Table 2).

Blood and serum analysis results:

Hematological and serum constituents' results showed no significant changes before and after vaccination or in urea and sulphur-fed or non-fed animal groups (Tables 1 and 2). The results also showed no variation from normal values in the vaccinated ewes which indicated that the injected vaccine was safe and did not affect blood, liver or kidney functions.

Antibody levels to CLA vaccines as detected by ELISA:

As shown in Table 3, vaccination resulted in a similar increase of antibody titres in all groups of ewes under experimentation, including the control group. Booster immunization resulted in little increase in the antibody titre at two weeks post first immunization as compared with the titres two weeks after the first immunization.

Birth Outcome:

It was observed that 2 ewes: one from group 1 and one from group 3 gave birth to paralyzed lambs. When these were post-mortemed, both had brain malacia (Fig. 2). This was possibly due to suphur in the feed.

FIGURES (Figure 1 and Figure 2):

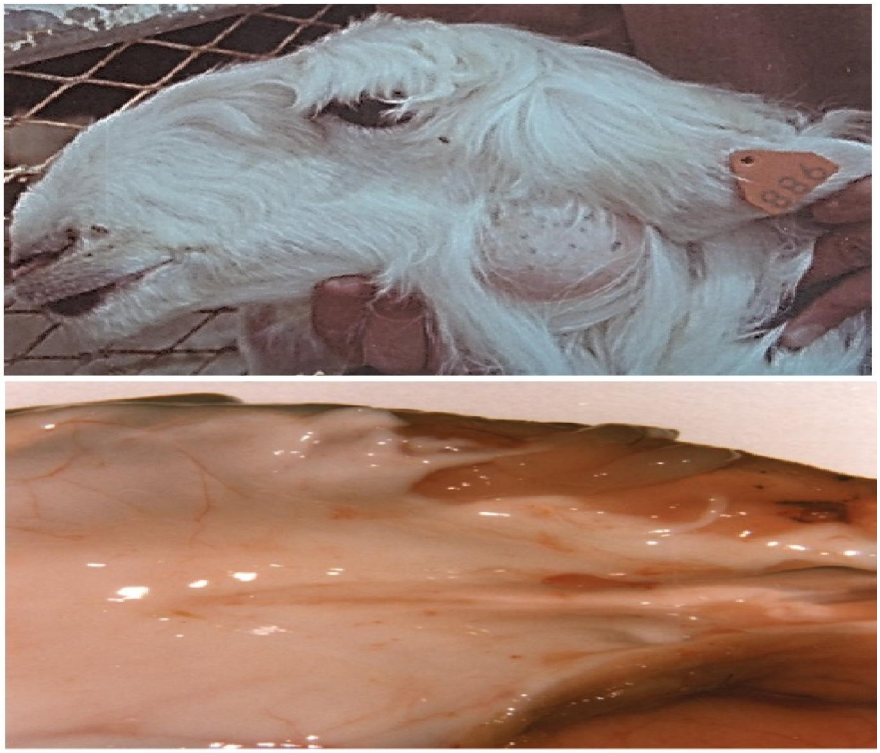

Fig. 1. Development of abscess on the neck of a ewe after feeding on urea for 3 weeks (Top).
Fig 2. Encephalomalacia (fluidly brain) in a lamb born from a ewe fed on sulphur (Bottom)

Table 1. Effect of dietary urea, urea plus sulphur or sulphur on the blood picture before, 2 weeks after vaccination and 2 weeks post booster with CLA disease vaccine

Animal Groups / Tests and periods	1 (urea) n=5	2 (urea+sulphur) (n=5)	3 (sulphur) (n=5)	4 (negative control) (n=5)
Hb (g/dl)				
Before feeding	9.8 ± 1.2	±1.4	±1.4	9.8 ±1.4
Before vaccination	10.2 ±1.2	9.6 ± 1.2	10.6 ± 1.6	9.8 ± 1.4
2 weeks after vaccination	10.2 ±0.9	10.0 ±1.4	11.0 ±1.4	10.4 ± 1.2
2 weeks post booster vaccination	10.0 ±1.2	8.9 ±1.4	10.6 ±1.2	9.6 ±1.6
PCV (l/l)				
Before feeding	0.30 ±0.02	0.30 ±0.03	0.34 ±0.02	0.28 ±0.06
Before vaccination	0.34 ± 0.04	0.32 ±0.02	0.34 ±0.02	0.030 ±0.02
2 weeks after vaccination	0.30 ±0.02	0.34 ±0.01	0.34 ±0.04	0.030 ±0.02
2 weeks post booster	0.30 ±0.02	0.32 ±0.02	0.32 ±0.02	0.032 ±0.04

Table 2. Effect of dietary urea, urea plus sulphur or sulphur on serum constituents before, 7 days after vaccination and 7 days post booster with CLA disease vaccine

Animal Groups / Tests and periods	1 (urea) n=5	2 (urea+sulphur) (n=5)	3 (sulphur) (n=5)	4 (negative control) (n=5)
AST (IU)				
Before feeding				
Before vaccination	14.2 ± 2.2	18.8 ±4.2	16.5 ±2.1	12.4 ±2.6
2 weeks after vaccination	10.8 ± 1.4	12.6 ±2.4	14.8 ±2.6	14.4 ±4.2
2 weeks post booster	16.2 ±2.4	16.8 ±3.4	14.8 ±2.6	10.8 ±3.4
	10.6 ±1.8	12.6 ±2.4	14. 4 ±2.2	14.4 ±2.4
Creatinine (µmol/l)				
Before feeding	68.4 ±4.8	54.6 ±4.4	48.8 ±4.6	62.2 ±4.6
Before vaccination	60.2 ± 3.4	60.2 ±2.2	52.6 ±3.2	62.2 ±1.2
2 weeks after vaccination	56.8 ± 6.6	56.4 ±4.2	56.2 ±2.6	54.8 ±2.6
2 weeks post booster	68.4 ±4.8	54.6 ±4.4	54.4 ± 4.2	61.2 ±2.2

Table 3. Antibody titres against C. pseudotuberculosis vaccine in sheep serum 1/20 diluted as detected by ELISA (Mean ±1SD)

Treatment groups / Periods of testing	Group 1	Group 2	Group 3	Group 4
Before vaccination	0.24 ±0.2	0.26 ±0.2	0.22 ±0.01	0.30 ±.02
2 weeks after vaccination	0.64 ±0.02	0.60 ±0.04	0.62 ±0.01	0.58 ±0.04
2 weeks after booster vaccination	0.70 ± 0.1	0.66 ±0.04	0.62 ±0.04	0.66 ±0.2

*Value = OD value of vaccinated animal serum – OD value of control well mean (no serum) Values ≥ 0.4 are considered positive.

4. Discussions

The dual effect of vaccination and oral feeding of urea and sulphur on the immunity of sheep against caseous lymphadenitis are less documented in the literature. This experiment was conducted to investigate some of the possible factors that affect the outcome of vaccination against CLA. Urea and sulphur are sometimes added to sheep rations to boost production in Saudi Arabia and in many other countries around the globe where poor quality feed is sometimes offered. Application of vaccination against CLA in Saudi Arabia is not yet effective as it should be in minimizing the incidence of CLA abscesses in sheep flocks. The real cause of such problem is not identified and questions have been raised to determine if the vaccine(s) itself is not effective or the application process does not follow the recommendations or there are other unforeseen causes.

Both increased urea in the blood and sulphur in the diet are suspected to have an effect on the immune system of the animal. Being very soluble, addition of urea to the animal feed at a concentration of 1 mg/kg is likely to make part of it absorbed to the blood of the ewes under experimentation. Feeding urea to ewes have resulted in some of them developing abscesses which indicated that their immunity was lowered compared to control sheep. This is attributed to a dysfunction of macrophages (Grimble and Grimble 1998). The dysfunction of macrophages in uremia has been ascribed to nitrous oxide (NO) production by macrophages in various tissues and organ systems. Nitrous oxide play many roles in the body such as control of systemic blood pressure, platelet aggregation, control of renal hemodynamics, regulation of renal tubular transport, immune defenses, and neuronal signaling in central and peripheral nervous systems (Narita et al., 1995; Lowenstein et al., 1994). In the area of immune defense mechanisms, NO, which is released by macrophages on induction with bacterial endotoxins, g-interferon, or tumor necrosis factor, is believed to function as the effector molecule mediating the cytotoxicity (Hibbs et al., 1988; Holan et al., 2006). The NO inhibits the iron-containing enzymes in the target cells, including several enzymes involved in mitochondrial respiration (Imai et al., 1993). In addition, NO also inhibits DNA synthesis in the target cells. Thus NO may mediate the macrophage-dependent killing of bacteria, protozoa, and fungi.

Our results showed neither urea or sulphur affected the level of antibodies produced by the CLA vaccine. Antibody levels against CLA vaccine were detected using ELISA test. In many studies, serological tests including ELISA assays have been set up (Menzies et al., 1994; Maki et al., 1985; Sutherland et al., 1987; Pepin et al., 1988), but often data are difficult to interpret. This is particularly true for chronically infected adult animals with old thick walled lesions where infection with Corynebacterium pseudotuberculosis is not always associated with a significant antibody response. Another difficulty arises from the persistence of antibodies in animals which have been in contact with Corynebacterium pseudotuberculosis and have eliminated it thereby producing false positives. This was faced in this study where antibodies were detectable in some non-vaccinated animals in levels with no significant differences with some vaccinated animals.

Enzyme linked immunosorbent assay (ELISA) tests have been developed to study the humoral response to the immunodominant PLD in experimentally infected sheep (Pépin et al., 1988, 1991; 1993 and 1997). Antibodies develop from the fifth day following challenge and reach a plateau approximately three weeks post-challenge after which the titre slowly decreases (Pépin et al. 1988, 1991; Pépin et al. 1993).

Some of the ewes fed on diet containing urea developed abscesses after 3 weeks from the start of the experiment, and that some ewes fed on diet containing sulphur gave birth to paralyzed lambs. This was attributed to lowered macrophage function in producing NO. It is postulated that some of urea might have been absorbed to the blood from the rumen, and that the sulphur might have interacted with the copper or vitamin B1 in the feed making them less available thus affecting the nervous tissue and also affect the immunity of the ewes under experimentation. Sulphur, if above normal will also cause brain malacia of the new born (Mahmoud et al., 1993).

In conclusion, feeding concentrate rations containing 1 gm/kg sulphur, urea or sulphur plus urea did not affect the general health of experimental animals. It also did not affect positively or negatively the immune response of the ewes to the administered CLA vaccine.

Acknowledgment:

Thanks to the Deanship of Scientific Research, Qassim University, and SABIC company for financial support. Thanks due to Prof. Osama Mahmoud, Department of Veterinary Medicine, College of Agriculture and Veterinary Medicine, Qassim University for his professional assistance.

Corresponding Author:
Dr. Khaled B. Alharbi
Department of Veterinary Medicine, College of Agriculture and Veterinary Medicine, Qassim University, P. O. Box 6622 Buraydah 51452, Qassim, Saudi Arabia
E-mail: kbhrby@qu.edu.sa

References

1. Grimble, R.F. “Sulphur amino acids, glutathione and immune function”. In: Caldor, P.C., Field, C.J., Gill, H.S. (eds): Nutrition and Immune Function. Wallingford, UK, CABI Publishing (2001): 133–150.
2. Grimble, R. F. and Grimble G. K. Immunonutrition: role of sulphur amino acids and ployamines. J. Nutrit. 14, (1998): 605 – 610.
3. Guthrie, H.A. Introductory Nutrition, 6th edn. St Louis, MO, Times Mirror/Mosby College Publishing, (1986):112-118.
4. Hibbs, J. B., Jr., R. R. Taintor, Z. Vavrin, and E. M. Rachlin. Nitric oxide: a cytotoxic activated macrophage effector molecule. Biochem. Biophys. Res. Commun. (1988) 157: 87–94, 1988.
5. Holan V, Pindjakova J, Krulova M, Neuwirth A, Fric J, Zajicova A. Production of nitric oxide during graft rejection is regulated by the Th1/Th2 balance, the arginase activity, and L-arginine metabolism. Transplantation. (2006):81:1708–15.
6. Imai, Y., H. Kolb, and V. Burkar. Nitric oxide production from macrophages is regulated by arachidonic acid metabolites. Biochem. Biophys. Res. Commun. (1993)197: 105–109.
7. Iyengar, R., D. J. Stuehr, and M. A. Marletta. Macrophage synthesis of nitrite, nitrate, and N-nitrosamines: precursors and role of the respiratory burst. Proc. Natl. Acad. Sci. USA (1987) 84: 6369–6373.
8. Jones, T. C., R. D. Hunt, King, N. W. Deficiency Diseases. In: Veterinary Pathology, 6th Edition. Williams and Wilkins. Baltimore, Philadelphia. (1997).
9. Keen, C.L, Uriu-Adams, J.Y, Ensuma, J.L & Gershwin, M.E. Trace elements/minerals and immunity. In Gershwin ME, Nestel P, Keen CL (eds): Handbook of Nutrition and Immunity. Totowa, NJ, Humana Press, (2004): 117–140.
10. Kidd, M.T. Minerals, disease and immune function. In Taylor-Pickard JA, Tucker, LA (eds): Re-defining Mineral Nutrition. Nottingham, UK, Nottingham University Press, (2005): 119–125.
11. Lee, J, Knowles S.O & Judson G.J. Trace element and vitamin nutrition of grazing sheep. In Freer M, Dove H (eds): Sheep Nutrition. Wallingford, UK, CABI Publishing, (2002): 285–311.

12. Lowenstein, C. J., J. L. Dinerman, and S. H. Snyder. Nitric oxide: a physiologic messenger. Ann. Intern. Med. (1994):120: 227–237.
13. Mahmoud, O. M., Haroun, E. M. and Sulman, A.. Encephalomyelomalacia in lambs drinking deep-bore water high in sulphur content. SVS, Edinburgh, UK, (1993):p.p. 205 - 206.
14. Maki, L.R., Shen, S.H., Bergstrom, R.C., Stetzenbach, L.D. Diagnosis of Corynebacterium pseudotuberculosis infections in sheep, using an enzyme-linked immunosorbent assay. Am. J. Vet. Res. (1985):46, 212-214.
15. McClure S.J. Mineral nutrition and effects on gastrointestinal immune function of sheep. Aust J Exp Agric (2003); 43: 1455–1462.
16. McKenzie R.C, Arthur J.R, Miller S.M, Rafferty T.S & Beckett G.J. Selenium and the immune system. In Caldor PC, Field CJ, Gill HS (eds): Nutrition and Immune Function. Wallingford, UK, CABI Publishing, (2001): 229–250.
17. Menzies, P.I., Muckle, C.A., Hwang, Y.T., Songer, G.J. Evaluation of an enzyme-linked immunosorbent assay using an Escherichia coli recombinant phospholipase D antigen for the diagnosis of Corynebacterium pseudotuberculosis infection. Small Rumin. Res. (1994):13, 193-198.
18. Narita, I., W. A. Border, M. Ketteler, and N. A. Noble. Nitric oxide mediates immunologic injury to kidney mesangium inexperimental glomerulonephritis. Lab. Invest. (1995): 72: 17–24.
19. Park S.Y, Birkhold S.G, Kubena L.F, Nisbet D.J & Ricke S.C. Review on the role of dietary zinc in poultry nutrition, immunity and reproduction. Biol Trace Element Res (2004); 101: 147–163.
20. Pepin, M., Pardon, P. and Lantier, F. Corynebacterium pseudotuberculosis infection in adult ewes by inoculation in the external ear. American Journal of Veterinary Research (1988):49, 459.
21. Pepin, M., Fontaine, J.J., Pardon, P., Marly, J., Parodi, A.L.Histopathology of the early phase during experimental Corynebacterium pseudotuberculosis infection in lambs. Vet. Microbiol. (1991):29, 123–134.
22. Pepin, M., Pardon, P., Marly, J., Lantier, F., Arrigo, J.L. Acquired immunity after primary caseous lymphadenitis in sheep. Am. J. Vet. Res. (1993):54, 873–877
23. . Pepin, M., Seow, H.F., Corner, L., Rothel, J.S., Hodgson, A.L.M,. Wood, P.R. Cytokine gene expression in sheep following experimental infection with various strains of Corynebacterium pseudotuberculosis differing in virulence. Vet. Res. (1997):28, 149–163
24. Prasad, A.S. Zinc, infection and immune function. In Caldor PC, Field CJ, Gill HS (eds): Nutrition and Immune Function. Wallingford, UK, CABI Publishing, (2001): 193–207.
25. Star, R. A. Southwestern Internal Medicine Conference: nitric oxide. Am. J. Med. Sci. (1993):306: 348–358.
26. Sutherland, S.S., Ellis, T.M., Mercy, A.R., Paton, M., Middleton, H. Evaluation of an enzyme-linked immunosorbent assay for the detection of Corynebacterium pseudotuberculosis infection in sheep. Aust. Vet. J(1987: 64, 263-266.
27. Underwood, E. J. Mineral Nutrition of Farm Animals. Common Agricultural Bureaux, UK. (1981).
28. Vallance, P., A. Loene, A. Calver, Collier, J. and Moncada S. Accumulation of an endogenous inhibitor of nitric oxide synthesis in chronic renal failure. Lancet (1992): 339: 572–575, 1992.

3/19/2014