

The Saga of Macrominerals and its Role in Reproduction in Domestic Animals: A Review

¹Rajesh Kumar, ²R.P. Diwakar, ³H.C. Verma, ⁴Anand Kumar,
⁵Pramod Kumar, ⁶K.D. Singh and ²Vibha Yadav

¹Department of Veterinary Gynaecology & Obstetrics, C.V.Sc. & A.H. and
Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya, UP, India

²Department of Veterinary Microbiology, C.V.Sc. & A.H. and UAT, Ayodhya, UP, India

³Department of Veterinary & Animal Husbandry Extension Education,
C.V.Sc. & A.H. and UAT, Ayodhya, UP, India

⁴Department of Veterinary Gynaecology & Obstetrics,
Ranchi Veterinary College, B.A.U., Kanke, Ranchi-06, Jharkhand, India

⁵Department of Veterinary Physiology & Biochemistry, C.V.Sc. & A.H. and UAT, Ayodhya, UP, India

⁶Department of ILFC, C.V.Sc. & A.H. and UAT, Ayodhya, UP, India

Abstract: A number of inorganic elements are essential for the growth, production and reproduction of animals. In addition, mineral requirements are also influenced by age, parity, stage of pregnancy and stage of lactation. Adequate quantity of minerals are utmost important for proper functioning of various physiological functions in animals. Deficiency and excess both have detrimental effects on production and reproduction; thus significantly affecting the economics of animal husbandry.

Key words: Domestic Animals • Macrominerals • Production • Reproduction

INTRODUCTION

A number of inorganic elements are essential for the growth, production and reproduction of animals [1]. In addition, mineral requirements are also influenced by age, parity, stage of pregnancy and stage of lactation. The inorganic elements are classified according to their requirements in animal body. Those required in greater quantities are referred as macrominerals (calcium, phosphorus, sodium, chloride, potassium, magnesium and sulfur). Macrominerals required in large amount each day and their concentration in diet is expressed as percentage of diet or in grams per kilogram of diet. Macrominerals has role in different physiological functions. Other elements required in much smaller amounts are referred as trace minerals (microminerals) i.e. copper, cobalt, iodine, iron, manganese, molybdenum, selenium, zinc, chromium and fluorine. Their concentration in diet is expressed as parts per million (ppm), which is equivalent to milligram per kilogram of diet or in some

cases as parts per billion (ppb), which is equivalent to microgram per kilogram of diet. Adequate quantity of minerals are utmost important for proper functioning of various physiological functions in animals. Deficiency and excess both have detrimental effects on production and reproduction; thus significantly affecting the economics of animal husbandry.

Role of Various Macro Minerals in Animal Reproduction

Calcium (Ca): Calcium is an essential component of skeleton and plays a pivotal role in maintaining homeostasis of vertebrates [2]. The maximum portion of calcium present in the bone as hydroxyapatite (99%) and small fractions present in the cell membrane or endoplasmic reticulum (0.9%), extracellular fluid or serum (0.1%) and in cytosol (0.00002%) [3]. Extracellular calcium exist in three forms i.e. ionized (50%), complexes to anions (5%) such as citrate, bicarbonate, phosphate, or lactate and protein bound (45%). Ionized calcium involved in array of physiological processes such as muscular

Corresponding Author: Rajesh Kumar, Department of Veterinary Gynaecology & Obstetrics, C.V.Sc. & A.H. and Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya, UP, India .

contraction, blood coagulation, enzyme activity, neural excitability, hormone secretion and cell adhesion [2]. In short, calcium serves two primary functions in the body namely maintaining structural integrity of bone and teeth and as a messenger or regulatory ion. There is high concentration gradient (10, 000 folds) between extracellular fluid and cytoplasm, which permits Ca^{2+} to function as a signaling ion to activate intracellular processes. The lipid bilayer of cell membrane has poor permeability to Ca^{2+} ; therefore, influx of Ca^{2+} into cytoplasm is controlled by heterogeneous group of calcium channels regulated by membrane potential, cell membrane receptors, or intracellular secondary messengers. Influx of Ca^{2+} into cells regulate cellular function (by interactions with a calcium binding protein, calmodulin; calcium sensitive protein kinase and G-protein linked Ca^{2+} sensing receptors in the cell membrane) and stimulate biological response (such as neurotransmitter release, contraction and secretion).

The normal reproductive process is compromised in case of severe calcium deficiency [4] possibly due to lack of tone of uterine muscle, as calcium sensitizes the tubular genital tract for action of hormones [5]. Majority of infertility problems in cattle [5] and buffaloes [6] are due to nutritional deficiency, calcium appears to affect reproduction indirectly because it affects the incidence of parturient paresis [7]. Plenty of available reports suggest that anestrus, sub-estrus and repeat breeding condition in cattle [8-12] and buffalo [6] might be due to calcium deficiency or imbalance. Stojkovic *et al.* [13] opined that GnRH induced LH release from pituitary is calcium dependent and in case of sub-threshold calcium level (calcium is lower than circulatory calcium) there is failure of LH release. Low level of calcium may increase the incidence of dystocia, retained fetal membranes, tardy uterine involution [14, 15], prolapse of vagina [16], cervix [17], uterus [14, 18, 19] and even rectum [20-22]. Hypocalcemia probably involve in pathogenesis of incomplete cervical dilation during parturition in ruminants [23]. Furthermore, hypocalcemia predisposes the cows for uterine diseases [24], has adverse effect on fertility in term of reduced conception rates and extended interval to pregnancy [25]. Moreover, Curtis *et al.* [15] studied the association between hypocalcemia and peri-parturient disorders and opined that loss of muscle tone associated with hypocalcemia could reduce normal uterine function and hypocalcemic cows have 3.2 times higher incidence of RFM than normal contemporaries. Hypocalcemia reduces muscle contraction, hence, rumen

function is compromised which results in in-appetence, thus, negative energy balance (NEB) ensues, consequently to cope up with NEB fat mobilization occur and the end result is fatty liver syndrome and ketosis [26], which further suppress appetite. Pharmacological induction of hypocalcemia by (IV infusion of Na_2EDTA) reduces the contractile strength and motility of rumen as well as the ruminal dysfunction may occur considerably before the onset of clinical signs of hypocalcemia in sheep [27]. Subclinical hypocalcemia compromises motility of digestive tract in bovines [28] as well as reduces insulin concentrations [29] which results in reduced milk yield and compromised fertility. In spite of hypocalcemia, hypercalcemia is also detrimental to fertility by causing secondary deficiency of other minerals by inhibiting their intestinal absorption [30, 31].

The animal ration preferably should have 0.75 to 0.80 per cent calcium on dry matter basis [32]. In order to maximize milk production and reproductive efficiency, prevention of milk fever should be of cardinal concern. Thus, the cows must be supplied with adequate amount of calcium. Hignett and Hignett [33] mentioned that a minimum calcium-to-phosphorus ratio of 1.5:1 and 2.5:1 for lactating cows is suitable for normal reproductive functioning.

Phosphorus (P): The normal values of phosphorus are 4-8 mg/dl [34], level below 4mg/dl usually indicate phosphorus deficiency [18, 34]. The phosphorus content should be 0.26% and 0.40% of the ration on a dry matter basis for dry cows and high producing dairy cows respectively [35]. Gerloff and Morrow [7] opined that phosphorus deficiency is most frequently associated with reproductive abnormality in cattle. It is necessary for normal energy and phospholipid metabolism as well as skeletal muscle development and milk production. Serum phosphorus level generally reflects intake although they were modified by vitamin D and calcium status. There is no consensus among scientists that hypophosphatemia is a cause of infertility syndrome in bovines [18]; some suggest that hypophosphatemia is associated infertility is characterized by anestrus, sub-estrus, irregular cycles, low conception rate [36-38] and repeat breeding syndrome in absence of other clinical sign of phosphorus deficiency, however, others reported no adverse effect on fertility [39]. Kumar *et al.* [23] observed the effect of GnRH and $\text{PGF}_{2\alpha}$ in anestrus crossbred cows and recorded non-significantly lower overall mean phosphorus level in untreated anestrus

cows than those of treated contemporaries, furthermore, the value was non-significantly higher in conceived cows as compared with non-conceived one (8.91 ± 0.27 vs 8.55 ± 0.17 mg/dl; $p > 0.05$).

Magnesium (Mg): Adequate supply of dietary magnesium important to safeguard animals health as it is an essential nutrient in animals [40]. All enzymatic reactions involving the utilization and formation of ATP have an absolute requirement for magnesium. It is indispensable for all phosphate transfer reactions, stabilizes the anionic charges on ATP, ADP and AMP, serves as a co-factor for thiamin pyrophosphate requiring reactions and participates in the synthesis of nucleic acid and the utilization of acetyl- CoA (CoA). Through its interaction with phosphoryl groups of membrane phospholipids, its modulation of ATP metabolism and its effect on trans-cellular calcium ion gating, Mg exerts many regulatory effects on cell membrane function, electrical conductivity and hormonal signaling of intracellular processes [41]. Magnesium is indispensable for maintaining normal bone growth, activating various enzyme system, nervous system and degradation of fiber in rumen [42]. Sikka [43] stated that magnesium requirements are more pronounced at the time of occurrence of high energy processes, as magnesium act as co-factor in all the ATP requiring enzymatic process in overall general metabolism, nevertheless reproduction is one of the most dominating process in biological system. In heifers, udder edema can be prevented by feeding 18 mg magnesium oxide 5-6 weeks prior to parturition and high magnesium is needed in early lactation to maintain normal milk output [42]. Moreover, Radostits *et al.* [44] opined that nutritional deficiency of magnesium causes lactation tetany in cows. Kumar *et al.* [23] studied the effect of GnRH and PGF_{2 α} in anestrus crossbred cows and recorded non-significantly lower overall mean magnesium level in untreated anestrus cows than those of treated contemporaries, furthermore, the value was non-significantly higher in conceived cows as compared with non-conceived one (3.84 ± 0.11 vs 3.46 ± 0.15 mEq/L; $p > 0.05$).

The relation between magnesium supply and milk fever is not extensively studied but any decrease responsiveness to an acute drop in plasma Ca was observed in hypomagnesemic cows [45]. Moreover, Van der Braak *et al.* [46], observed slower mobilization of Ca in cows fed magnesium deficient ration during the dry period. Lean *et al.* [47] opined that increase magnesium content in prepartal ration significantly reduced the

incidence of milk fever. Furthermore, Goff [48] postulated that sensitivity of PTH receptors is decreased in hypomagnesemic cows (PTH is an important hormone for calcium homeostasis). Thus, in order to get optimum production and reproduction, ration must be supplemented with adequate amount of Mg to eschew the deficiency.

Sodium and Chloride (Na and Cl): As in case for most other nutrients, dietary requirements for Na and Cl increases significantly during lactation [41]. The sign of Na or Cl deficiency have been well documented in cattle and include lethargy, anorexia, weight loss, hypogalactia, neuromuscular and cardiovascular dysfunction, depraved appetite [49-52] and compromised renal function [51-53]. Thus, sodium and chloride deficiency indirectly affect the reproduction. Moreover, Aines [54] studied the effect of withheld of sodium chloride for long time in dairy cattle and observed that effect becomes very evident after calving. Lactation caused rapid loss of weight because the appetite was poor. Milk secretion declined and ceased. The cows become cachectic and failed to estrum. On per rectal examination ovaries were found to be small and inactive; an occasional small 3/8 to 1/2 inch follicle was palpated. Estrum did not return until salt was supplied and the appetite, nutritive state and body condition improved. For dairy cattle, salt requirement can be easily met by adding 1% salt to the grain and availability of salt as free lick [42].

Potassium (K): Potassium is major intracellular cation of the body. The physiological functions of potassium are maintenance of electrolyte balance, enzyme activator and proper functioning of muscle and nerve. Moreover, potassium is required for normal secretion of insulin. The deficiency of potassium is characterized by weakness of muscles, musculature of female genital tract, anoestrus, cyclic irregularity, long inter-calving intervals in cows and still born or weak expelled calves [42]. Furthermore, Excessive dietary potassium is a major factor increasing the susceptibility of dairy cattle to severe hypocalcemia at calving [1]. The potassium absorbed from the diet induces a mild metabolic alkalosis, which interfere the ability of tissue to recognize PTH, thereby interfering with calcium homeostasis, which might have an indirect effect on reproduction.

Sulfur (S): About 0.15 % of the body weight is the sulfur. The sulfur is found in chondroitin sulfate and amino acids methionine, cysteine (cystine), homocysteine and taurine

and in the B vitamins thiamine and biotin. Indeed, a sulfur deficiency is deficiency in the sulfur containing amino acids, thiamine, or biotin [1]. Ruminants needed certain amount of dietary sulfur to provide the rumen microbes with the material needed to synthesize the cysteine, methionine, thiamine and biotin that in turn used by ruminants.

CONCLUSION

Mineral has an important bearing in reproduction of domestic animals. Deficiency as well as excess of the minerals results in compromised reproductive function. In order to maximize reproductive efficiency and to get optimum economic return, the ration of animal should contain adequate quantity of minerals. Furthermore, it is suggested that area specific mineral mixture should be made available in a particular area to prevent any deficiency of mineral in domestic animals.

REFERENCES

- Goff, J.P., 2005. Minerals. In: William O. Reece (Ed). Dukes' Physiology of Domestic Animals. (12th ed., pp: 575-599). Panima Publishing Corporation New Delhi/Bangalore.
- Capen, C.C. and T.J. Rosol, 2003. The calcium regulating hormones: Parathyroid hormone, Calcitonin and Cholecalciferol. In: M.H. Pineda and M.P. Doole (Eds). McDonald's Veterinary Endocrinology and Reproduction (5th ed., pp: 71-140). A Blackwell Publishing Company, USA.
- Rosol, T.J., D.J. Chew, L.A. Nagoda, C.C. Capen, 1995. Pathophysiology of calcium metabolism. Vet. Clin. Pathol., 24: 49
- Youngquist R.S. and W.R. Threlfall (Ed.), 2007. Current Therapy in Large Animal Theriogenology. (2nd ed.). Published by the author. Distributed by Saunders, St. Louis, Missouri.
- Kumar, R., M.G. Butani, F.S. Kavani and A.J. Dhami, 2020. Hormonal Interventions to Augment fertility and its Effect on Blood Biochemical Profile in Crossbred Cows. Haya Saudi J. Life Sci., 5(9): 176-181. DOI: 10.36348/sjls.2020.v05i09.005.
- Butani, M.G., A.J. Dhami and R. Kumar, 2011. Comparative blood profile of progesterone, metabolites and minerals in anoestrus, suboestrus, repeat breeding and normal cyclic buffaloes. Indian J. Field Vets., 7(2): 20-24.
- Gerloff, B.J. and D.A. Morrow, 1986. Effect of nutrition on reproduction on Reproduction in Dairy Cattle. In D.A. Morrow (Ed.). Current therapy in theriogenology 2, Diagnosis, treatment and prevention of reproductive diseases (2nd ed. pp: 310-320). W.B. Saunders Company, USA.
- Kumar, R., 2008. Studies on major infertility problems their diafnosis and therapeutic management in crossbred cows under field conditions. M.V.Sc. Theis. AAU, Anand, Gujarat, India.
- Kumar, R., M.G. Butani, A.J. Dhami, F.S. Kavani, M.D. Patel and R.G. Shah, 2009. Progesterone, metabolites and minerals in anestrus, subestrus, repeat breeding and cyclic cows. Indian J. Anim. Reprod., 30(2): 19-22.
- Kumar, R., M.G. Butani, A.J. Dhami, F.S. Kavani and R.G. Shah, 2009. Effect of different therapies on fertility and serum progesterone, metabolites and minerals profile in repeat breeding crossbred cows. Indian J. Field Vets., 5(2): 1-8.
- Kumar, R., M.G. Butani, A.J. Dhami, F.S. Kavani, R.G. Shah and A. Killedar, 2011. Management of anoestrus and suboestrus cows using hormonal and nonhormonal drugs. Indian J. Anim. Reprod., 32(1): 24-27.
- Kumar, R., M.G. Butani, F.S. Kavani, A.J. Dhami and R.G. Shah, 2013. Influence of hormonal and nonhormonal therapies on fertility and serum progesterone, metabolites and minerals profile in anoestrus crossbred cows. GAU Res. J., 38(2): 112-118.
- Stojilković, S.S., J.P. Chang, D. Ngo, K. Tasaka, S. Izumi and K.J. Catt, , 1989. Mechanism of action of GnRH: the participation of calcium mobilization and activation of protein kinase C in gonadotropin secretion. J. Steroid Biochem., 3(4B): 693-703.
- Morrow, D.A., 1980. The role of nutrition in dairy cattle reproduction. In: Current Therapy in Theriogenology. Morrow D. A. (Ed.), WB Saunders Company, Philadelphia, pp: 449.
- Curtis, C.R., H.N. Erb, C.J. Sniffen, R.D. Smith, P.A. Powers, M.C. Smith, M.E. White, R.B. Hillman and E.J. Pearson, 1983. Association of parturient hypocalcemia with eight periparturient disorders in Holstein cows. Journal of the American Veterinary Medical Association, 183(5): 559-561.
- Kumar, R., B. Singh, S. Husain, R.P. Diwakar and H.C. Verma, 2020. Therapeutic Management of Pre-Partum Vaginal Prolapse in a Crossbred Cow: A Case Report. International Journal of Livestock Research, 10(6): 164-167.

17. Parikh, S.S., R.B. Makwana, B.D. Savaliya, T.K. Patbandha and R. Kumar, 2018. Pre-partum cervicovaginal prolapse in Gir cow. *Journal of Pharmacognosy and Phytochemistry*, 4 (SI): 238-240.
18. Noakes, D.E., T.J. Parkinson and G.C.W. England, 2019. *Veterinary Reproduction and Obstetrics*. (10th Ed.) Saunders Ltd.
19. Singh, B., K.P. Singh, R. Kumar, S.V. Singh and S. Husain, 2020. Postpartum Uterine Prolapse in a Goat and its Successful Management. *Ind. J. Vet. Sci. and Biotech.*, 16(1): 73-74.
20. Patel, A., R. Kumar, R.K. Verma, R. Kumar and C. Gnagwar, 2018. Management of Pre-Partum Recto-Vaginal Prolapse in a Cow. *Int. J. Curr. Microbiol. App. Sci.*, 7(SI): 1244-1247.
21. Kumar, R., D.K. Yadav, V.K. Yadav, S. Jaisawal, S. Srivastava and S. Gautam, 2017. Recto-cervico-vaginal prolapse in non-descript postpartum buffalo and its clinical management. *Bull. Env. Pharmacol. Life Sci.*, 7(2): 46-50.
22. Kumar, R., R.P. Diwakar, Ramakant, S. Husain and K. Alam, 2020. A rare case of antepartum rectal prolapse in a goat and its management: A case report. *Journal of Entomology and Zoology Studies*, 8(2): 1285-1287.
23. Kumar, R., B. Singh, R.P. Diwakar, P. Kumar, H.C. Verma and S. Husain, 2020. Therapeutic Management of Incomplete Cervical Dilatation In A Buffalo: A Case Report. *Journal of Animal Feed Science and Technology*, 8(1): 29-31.
24. Whiteford, L.C. and I.M. Sheldon, 2005. Association between clinical hypocalcaemia and postpartum endometritis. *Vet. Rec.*, 157: 202-203.
25. Goshen, T. and N.Y. Shpigel, 2006. Evaluation of intrauterine antibiotic treatment of clinical metritis and retained fetal membranes in dairy cows. *Theriogenology*, 66(9): 2210-2218. <https://doi.org/10.1016/j.theriogenology.2006.07.017>
26. Boland, M.P., P. Lonergan and O. Callaghan, 2001. Effect of nutrition on endocrine parameters, ovarian physiology and oocyte and embryo development. *Theriogenology*, 55: 1323-1340.
27. Huber, T.L., R.C. Wilson, A.J. Stattelmann and D.D. Goetsch, 1981. Effect of hypocalcemia on motility of the ruminant stomach. *Am. J. Vet. Res.*, 42(9): 1488-90. PMID: 7325457.
28. Hara, S., Y. Ikegaya, R.J. Jørgensen, J. Sasaki, M. Nakamura and N. Tomizawa, 2003. Effect of Induced Subclinical Hypocalcemia on the Motility of the Bovine Digestive Tract. *Acta Vet Scand*, 44: 251. <https://doi.org/10.1186/1751-0147-44-S1-P76>.
29. Goff, J.P., 1999. Dry cow nutrition and metabolic disease in parturient cows. *Proceeding Western Canadian Dairy Seminar Red Deer*.
30. Hurley, W.L. and R.M. Doane, 1989. Recent developments in the roles of vitamins and minerals in reproduction. *J. Dairy Sci.*, 72: 784-804.
31. Saba, L., Z. Bialkowski and S. Wojcik, 1987. Evaluation of mineral nutrition of milk cows in the period between pregnancies. *Pol. Arch Vet.*, 25: 237-246.
32. Schweigert, F.J. and H. Zucker, 1988. Concentration of vitamin A, beta-carotene and vitamin E in individual bovine follicles of different quality. *Journal of Reproduction and Fertility*, 82: 575-579.
33. Hignett, S.L. and P.G. Hignett, 1953. The influence of nutrition on reproductive efficiency in cattle. *Veterinary Records*, 65: 21.
34. Roberts, S.J., 1986. *Veterinary Obstetrics and Genital Diseases*. 3rd Ed. N.Y. Ithaka.
35. NRC, 1978. *National Research Council-National Academy of Sciences; Nutrient requirement of dairy cattle*. Washington, D.C., National Academy of Sciences, pp: 1-76.
36. Hignett, D.L. and P.G. Hignett, 1951. The influence of nutrition on reproductive efficiency in cattle. The effect of calcium and phosphorus intake on the fertility of cows and heifers. *Vet. Rec.*, 63: 603-609.
37. Morrow, D.A., 1969. Phosphorus deficiency and infertility in dairy heifers. *J. Am. Vet. Med. Assoc.*, 154: 761.
38. Morris, R.S., 1976. *Diagnosis of infertility in large dairy herds*. Sydney: Refresher course for Veterinarians. Proc No 28.
39. Carstairs, J.A., D.A. Morrow and R.S. Emery, 1980. Postpartum reproductive function of dairy cows as influenced by energy and phosphorus status. *J. Anim. Sci.*, 51: 1122-1130.
40. Schonewille, J.Th., 2013. Magnesium in dairy cow nutrition: an overview. *Plant Soil.*, 368: 167-178. DOI 10.1007/s11104-013-1665-5.
41. Fettman, M.J., 2001. Calcium, Phosphorus and other macroelements. In: H. Richard Adams (Ed) *Veterinary Pharmacology and Therapeutics* (8th ed., pp: 722-743). Iowa State University Press, Iowa.
42. Ahuja, A.K. and D. Parmar, 2017. Role of Minerals in Reproductive Health of Dairy Cattle: A Review. *International Journal of Livestock Research*, 7(10): 16-26.
43. Sikka, P., 1992. Role of Minerals in Reproduction. *Indian J. Dairy Science*, 45: 159-167.

44. Radostits, O.M., C.C. Gay, K.W. Hinchcliff and P.D. Constable, 2007. *Veterinary Medicine. A text Book of the disease of Cattle, Horses, Sheep, Pigs and Goats*. 10th ed. Saunders, Philadelphia, USA.
45. Contreras, P.A., R. Manston and B.F. Sansom, 1982. Calcium mobilisation in hypomagnesaemic cattle. *Res. Vet. Sci.*, 33: 10-16.
46. Van Der Braak, A.E., A.T. Van't Klooster and A. Malestein, 1987. Influence of a deficient supply of magnesium during the dry period on the rate of calcium mobilisation by dairy cows at parturition. *Res. Vet. Sci.*, 42: 101-108
47. Lean, I.J., P.J. DeGaris, D.M. McNeil and E. Block, 2006. Hypocalcemia in dairy cows: meta-analysis and dietary cation anion difference theory revisited. *J. Dairy. Sci.*, 89: 669-684.
48. Goff, J.P., 2008. The monitoring, prevention and treatment of milkfever and subclinical hypocalcemia in dairy cows. *Vet. J.*, 176: 50-57.
49. Aines, P.D. and S.E. Smith, 1957. Sodium versus chloride for the therapy of salt deficient dairy cows. *J. Dairy Sci.*, 40: 682-688.
50. Fettman, M.J., L.E. Chase, J. Bentinck-Smith, C.E. Coppock and S.A. Zinn, 1984. Effect of dietary chloride restriction in lactating dairy cows. *J. Am. Vet. Med. Assc.*, 185: 167-172.
51. Fettman, M.J., L.E. Chase, J. Bentinck-Smith, C.E. Coppock and S.A. Zinn, 1984. Restricted dietary chloride with sodium bicarbonate supplementation for Holstein cows in early lactation. *J. Dairy Sci.*, 67: 1457-1467.
52. Fettman, M.J., L.E. Chase, J. Bentinck-Smith, C.E. Coppock and S.A. Zinn, 1984. Nutritional chloride deficiency in early lactation Holstein cows. *J. Dairy Sci.*, 67: 2321-2335
53. Whitlock, R.H., M.J. Kesler and J.B. Tasker, 1975. Salt (sodium) deficiency in dairy cattle: polyurea and polydipsia as a prominent clinical feature. *Cornell Vet.*, 65: 512-526.
54. Aines, P.D. Jr., 1954. An evaluation of sodium chloride deficiency in relation to the requirement of dairy cattle for salt. Thesis, Cornell Univ., Ithaca, N.Y.