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RESEARCH ARTICLE

QUALITY ASSESSMENT OF MINERAL MIXTURE SUPPLEMENTS FOR DAIRY ANIMALS IN SONEPAT DISTRICT (HARYANA).

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Abstract

Balanced nutrition is very essential for keeping animal body in good condition and to maintain their optimum production. Similarly trace minerals also play critical roles in the proper functioning of body enzymes, hormones and cells. As feed and fodder fed to the dairy animals do not provide all the minerals in the required quantity, supplementation with adequate amount of good quality mineral mixture in their ration is always needed to fulfil their daily need, even level of minerals in feed and fodder varies from region to region, thus mineral availability to the animal also varies. It is necessary to provide region specific mineral mixture accordingly. Requirement for minerals is influenced by several factors including age, stage of pregnancy and stage of lactation. Mineral deficiency may lead to infertility in cattle which accounts for major economic losses in dairy farming. In India feed and mineral manufacturing is largely an unregulated industry, no standard specification guidelines are followed to scientifically prepare good quality mineral mixtures which may provide the optimal nutrition to dairy animals. Keeping in view of this fact, the present study is envisaged to evaluate the concentration of macro and micro mineral quantitatively on various branded products being fed to the livestock in different regions of Sonepat district of Haryana.

Introduction:-

Feeding of the livestock is most important as it contributes 60 to70% of the total cost of rearing and milk productivity. Thus, quality feed and feed ingredients are of prime importance. One of the most common ingredients in animal feeding is mineral mixture. It is important because minerals play an important role in health, reproduction and production of livestock.

Mineral deficiency and metabolic diseases in all categories of dairy livestock have been reported due to lower content and low bioavailability of some essential macro and micro mineral in different feedstuffs (1). Delayed onset of puberty, silent or irregular estrus in heifers, anoestrus and long inter calving period in cows are reported to be some of important clinical manifestation exhibited by the animals from phosphorus deficient areas. Copper, Potassium and Sodium are related to reproduction in animals, whereas, Zinc is known to be essential for proper sexual maturity, more specifically with onset of estrus in female. Selenium deficient animals are reported to be more prone for the incidence of retained placenta, Manganese deficiency cause poor fertility problem in male and female. Delay in onset of puberty, delay uterine involution and decreased conception rate and sub optimal conditioning of

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the offspring are noted due to Cobalt deficiency. Chromium is known to improve the energy balance which in early lactation leads to improved reproduction. Milking animals should always be provided adequate amounts of calcium to maximize production and minimize health problems (2). Thus, it is evident that the minerals that affect reproduction in cattle are generally found within the trace element group, it is essential to feed the proper recommended level of minerals and vitamins to achieve optimum performance in terms of growth, reproduction and milk production. As feed and fodder fed to the dairy animals do not provide all the minerals in the required quantity, supplementation with adequate amount of good quality mineral mixture in their ration is always needed to fulfil their daily need, even level of minerals in feed and fodder varies from region to region, thus mineral availability to the animal also varies. It is necessary to provide region specific mineral mixture accordingly. In India by in large no regulatory mechanism is being followed by the manufacturing industries on standard specification so as to prepare scientifically good quality of mineral mixture.

Dairy animals of villages of district Sonipat, Haryana also suffers from infertility and low milk yield despite being availability of different brands mineral mixtures in the area apart from rich supply of green and dry fodder. A research to investigate the quality of different types of mineral mixtures available in tehsil Gohana of district Sonipat was undertaken by Ayurvet Research Foundation R&D Centre with the objective of matching the composition of standard specification guidelines and to cross check the authenticity of label claim. Outcome of the study will be used in creating mass awareness among the farmers and dairy owners of the region about benefit of balanced rationing with proper supplementation of good quality mineral mixtures to tackle the issue of infertility and sub optimal productivity.

Materials and methods:-

Sample collection:

Ten samples of commonly prevalent mineral mixture were collected from Veterinary medicine shops of different areas of Sonepat district and stored at room temperature before analysing for different parameters.

Apparatus:

Agilent High-performance liquid chromatography (HPLC) was used for the estimation of vitamin A, D3 and E. Harrison's Flame photometer was used for estimation of Calcium, Sodium and Potassium. Phosphorus was analyzed using Shimadzu UV- spectrophotometer. Cobalt, Chromium, Copper, Iron, Manganese, Magnesium, Selenium, Zinc were evaluated on Perkin Elmer's Atomic Absorption Spectrophotometer. Samples were digested under closed system in Anton Paar's Microwave Digestion System to rule out any error in results due to evaporation.

Reagents and Material:

Chemicals and reagents used were of analytical reagent grade and procured from authentic sources.

Estimation of Calcium by flame photometer: Sample were digested with concentrated hydrochloric acid for 10 minutes, cooled and then prepared in HPLC grade water then after filtered the solution before subjecting to flame photometer. Analytical grade Calcium carbonate was used as standard. The results were calculated using linear regression curve plot.

Estimation of Sodium and Potassium by flame photometer:

Prepared the sample by dissolving known weight in known volume of HPLC grade water and then filtered the solution before subjecting to flame photometer. Analytical grade sodium chloride and potassium chloride were used as standard. The results were calculated using linear regression curve plot.

Estimation of Phosphorus by UV spectrophotometer:

Sample were digested with concentrated sulfuric acid, cooled and added known volume of nitric acid and boiled till colorless solution was obtained then added molybdovanadate reagent to develop the color and took UV absorbance at 430 nm. Potassium dihydrogen phosphate was used as standard and the result were calculated using linear regression curve plot.

Estimation of Vitamin A by HPLC:

The solutions of the samples were prepared as per standard procedure given in IP (4) and carried out the analysis against reference standard on High Performance Liquid Chromatography (AGILENT, quaternary pump with PDA

2996 detector, USA) using C18 column (250 mm x 4.6 mm, 5 µm). Separations of the peaks were best obtained using filtered and degassed isocratic mixture of methanol and water in a ratio of 95:05 v/v at flow rate of 1.0 ml/min and detection at 325 nm. The results were calculated using linear regression curve plot.

Estimation of Vitamin D3 and Vitamin E by HPLC: Mineral mixture samples of 100 gm each were weighed accurately and transferred to 100-ml volumetric flask separately, there after added 75 ml of HPLC grade methanol for Vitamin D3 and acetonitrile (ACN) for Vitamin E and sonicated for 15 minutes, made up the volume with HPLC grade methanol and ACN respectively, filtered the resulting solution through 0.45µm filter. Carried out the analysis of samples against reference standard on High Performance Liquid Chromatography (AGILENT, quaternary pump with PDA 2996 detector, USA) using C18 column (250 mm x 4.6 mm, 5 µm). Separations of the peaks were best obtained using filtered and degassed isocratic mixture of methanol and water in a ratio of 98:02 v/v at flow rate of 1.0 ml/min and detection at 265 nm for Vitamin D3 and 285 nm for Vitamin E respectively. The results were calculated using linear regression curve plot.

Estimation of Copper, Cobalt, Chromium, Iron, Magnesium, Manganese, Selenium and Zinc:
Preparation of standard solution:
Prepared the stock solutions of standards in 0.5M HNO_3 acid and diluted further to make 10 ppm to 1 ppm dilutions. Used 0.5 M Nitric acid as a blank for standards. Samples were digested in Microwave Digestion System (MDS) and known volumes were prepared and filtered before subjecting to Atomic Absorption Spectrophotometer (AAS).

Preparation of sample solution:
Weighed accurately 250.0 mg of sample, transferred to sample vessel of MDS, added 8 ml nitric acid (70.0%) and 2ml of Hydrofluoric acid (40.0%), adjusted the vessels in the disc and selected programmed method, after completion of the digestion cooled it for 15 minutes and transferred the solution to 50 ml volumetric flask, adjusted the final volume with HPLC grade water.

Preparation of blank solution:
Digested 8 ml nitric acid (70.0%) + 2ml of Hydrofluoric acid (40.0%), made up the volume to 50 ml with HPLC grade water.

Analytical procedure:
Inserted the desired lamp in the instrument and prepared the method on software for respective element using Syngistix software of AAS. Calculated the result using linear regression curve plot.

Result and discussion:-

At least 17 minerals namely calcium, phosphorus, sodium, chlorine, potassium, magnesium, sulphur, cobalt, copper, iodine, iron, manganese, molybdenum, selenium, zinc, chromium and fluorine and three vitamins are required in dairy animals' diet for maintenance, proper growth performance, reproductive performance, optimal milk production and herd health. In villages, where there is lack of awareness regarding balanced rationing, supplementation with mineral mixture becomes most important and relevant. Number of manufacturers supply their product compromising with the quality. ICAR has mentioned in its guidelines (3) about the minimum requirement of minerals and vitamins for maintenance, pregnancy and lactation of the animal. This requirement mostly comes from scientifically designed balanced rationing and deficiency of any of them may be achieved through supplementation with mineral mixture (3).

Comparison of composition of all brands available with the standard guidelines (Table 1) revealed no set pattern / ratio of minerals and vitamins added. Vitamins were found to be mixed with mineral mixture, they are prone to lose their potency over passage of time in combination with different ingredients of mineral mixture. It is suggested that different packaging of vitamins should mention on label to mix it with rest of the ingredients at the time of use. Only one brand was found to provide the separate packaging for vitamins inside the main packet. Non-region-specific mineral mixture composition may not produce the desired result as livestock of different geographical region of country suffer from deficiency of different types of minerals and vitamins in their feed stuff. It is advisable to manufacture the area specific mineral mixture to meet the daily requirement of animal belonging to particular area, only one brand was observed to be providing area however in this study specific mineral mixture.

Common parameters were considered to be included in the study which were either present in one or other samples (table 2). Calcium was found to be present in range of 72.55% - 108.3% in all samples whereas, Phosphorus was found to be absent in three brands, rest were found to contain 81.33% - 94.27% of it. Magnesium was found to be present in range of 35.0% - 149.6%, Cobalt in range of 60.0% to 1200%, Copper in range of 26.0% - 108.3%, Zinc in range of 50.0% to 188.0%, Manganese in range of 68.0% - 171.3% less to 71.33% more, Iron in range of 40.0% - 126.3%, Selenium was found to be absent where label claim was made but present in brands with no claim. Chromium was observed to be in range of 0.33% to 257.0% of label claim. Sodium and potassium were found to be absent in all brands but one. Vitamin A was found to be in range of 11.7% - 111.0%, Vitamin D_3 absent – 121.31% and E absent to 90.44% of label claim.

Table 1:-Comparison of dosage per day of various elements and vitamins recommended for maintenance of diary animals weighing 400kg as per ICAR guidelines with the quantity available in samples analysed.

Minerals	**Maintenance requirement (per day)**	**Sample 1 30 g /d**	**Sample 2 50 g/d**	**Sample 3 50 g/d**	**Sample 4 40g/d**	**Sample 5 50 g/d**
Calcium (g)	**18 gm**	7.5 gm	12.5	10.625	8.5	13.25
Phosphorus (g)	**8 g**	3.75g	6.375	5.3125	4.25	5
Magnesium (g)	**8 g**	0.18g	0.3	0.25	0.2	0
Cobalt (mg)	**1.1 mg**	4.5 mg	7.5	6.25	5	0
Copper (mg)	**100 mg**	36	60	175	150	100
Zinc (mg)	**400**	288	480	400	320	700
Manganese (mg)	**150**	54	75	62.5	80	150
Iron (mg)	**500**	45	75	62.5	50	0
Selenium (mg)	**2.5**	.3mg	0	0.42	0	0
Vitamin A (I.U)	**44000 IU/DAY**	21000	35000	29166.66	38333.33	35000
Vitamin D3 (I.U)	**6000 IU/DAY**	4200	3500	2916.66	7666.66	7000
Vitamin E (I.U)	**1000 IU/DAY**	9	12.5	9.375	29.25	11.25

Minerals	**Maintenance requirement (per day)**	**Sample 6 30g/d**	**Sample 7 50g/d**	**Sample 8 28g/d**	**Sample 9 50 g/d**	**Sample 10**
Calcium (g)	**18 gm**	7.65	12.75	6.72	13.5	10.625
Phosphorus (g)	**8 g**	3.825	6.375	2.52	3.375	5.3125
Magnesium (g)	**8 g**	0.18	0.3	0	0	0.25
Cobalt (mg)	**1.1 mg**	4.5	7.5	5.6	5	6.25000
Copper (mg)	**100 mg**	36	250	28	250	175
Zinc (mg)	**400**	288	490	0	0	400
Manganese (mg)	**150**	45	120	33.6	0	62.5

Iron (mg)	**500**	45	75	168	500	62.5
Selenium (mg)	**2.5**	0	0.5	0	1	0.42
Vitamin A (I.U)	**44000 IU/DAY**	21000	37500	0	30000	29167
Vitamin D3 (I.U)	**6000 IU/DAY**	2100	8500	0	6000	2917
Vitamin E (I.U)	**1000 IU/DAY**	6.75	43.875	0	7.5	11.42

Table 2:-Analysis of different parameters and comparison with label claims. (*n=3)

Ingredient	**Sample-1**			**Sample-2**			**Sample-3**			**Sample-4**			**Sample-5**		
	Claimed, %	**Actual, % (mean)**	**% wrt claimed**	**Claimed, %**	**Actual, % (mean)**	**% wrt claimed**	**Claimed, %**	**Actual, % (mean)**	**% wrt claimed**	**Claimed, %**	**Actual, % (mean)**	**% wrt claimed**	**Claimed, %**	**Actual, % (mean)**	**% wrt claimed**
Calcium	25	26	104	25.5	27	105.8	25.5	18.5	72.55	25.5	22.32	87.52	26.5	24.9	93.96
Potassium	12.5	11.66	93.28	12.75	12.02	94.27	0.01	ND	Nil	0.01	ND	Nil	10	8.71	87.1
Magnesium	0.6	0.64	106.66	0.6	0.21	35	0.5	0.748	149.6	0.6	0.71	118.3	NA	0.991	+
Sodium	NA	ND	Nil	0.005	ND	Nil	0.0005	ND	Nil	0.0005	ND	Nil	NA	ND	Nil
Potassium	NA	ND	Nil	0.01	ND	Nil	0.01	ND	Nil	0.01	ND	Nil	NA	ND	Nil
Cobalt	0.015	0.074	493.33	0.015	0.035	233.3	0.0125	0.0311	248.8	0.015	0.009	60.0	NA	0.0017	+
Copper	0.12	0.13	108.33	1.2	1.0	83.33	4.5	1.4	31.11	5	1.3	26.0	2	1.2	60.0
Zinc	0.96	1.20	125	NA	2.02	+	0.8	1.29	161.3	0.98	0.3	30.6	1.4	2.63	187.85
Manganese	0.18	0.197	109.44	0.15	0.257	171.3	0.2	0.167	83.5	0.24	0.163	67.91	0.30	0.30	100.05
Iron	0.15	0.134	89.33	1.5	0.75	50.0	1.5	1.58	105.3	1.5	1.38	92.0	NA	0.71	+
Selenium	0.001	ND	Nil	NA	ND	Nil	NA	0.00057	+	0.001	ND	Nil	NA	0.006	+
Chromium	NA	0.002	+	NA	0.002	+	0.01	0.005	50.0	0.3	0.001	0.33	0.007	0.018	257.14
Vit. A (IU)	700000	780000	111.42	700000	404700	57.8	1150000	134900	11.73	1150000	809400	70.38	700000	269800	38.54
Vit. D3 (IU)	140000	169837	121.31	70000	4007	5.72	230000	ND	Nil	230000	ND	Nil	140000	NA	Nil
Vit. E (IU)	300	262.22	87.40	277	250.66	90.4	1083	668.4	61.71	1083	ND	Nil	277	205	74.0

Ingredient	Sample-6			Sample-7			Sample-8			Sample-9			Sample-10		
	Claimed, %	Actual, % (mean)	% wrt claimed	Claimed	Actual, % (mean)	% wrt claimed	Claimed	Actual, % (mean)	% wrt claimed	Claimed	Actual, % (mean)	% wrt claimed	Claimed	Actual, % (mean)	% wrt claimed
Calcium	22.74	17.2	75.63	24	26	108.33	27	25	92.60	25.5	26	101.96	25.5	27	105.88
Potassium	NA	6.66	+	9	7.32	81.33	0.06	ND	Nil	12.75	11.14	87.37	12.75	10.41	81.64
Magnesium	0.96	0.55	57.29	NA	0.25 l	+	NA	0.66 l	+	0.6	0.49	81.66	0.5	0.69	138.0
Sodium	NA	ND	Nil	NA	3.30	+	NA	ND	Nil	0.01	ND	Nil	0.01	ND	Nil
Potassium	0.01	ND	Nil	NA	ND	Nil	NA	ND	Nil	0.01	ND	Nil	0.01	ND	Nil
Cobalt	NA	0.0009	+	0.02	0.036	180.0	0.01	0.0017	17.0	0.015	0.18	1200.0	0.0125	0.006	48.0
Copper	NA	1.5	50.0	0.1	0.05	50.0	0.5	0.22	44.0	0.12	0.09	75.0	0.35	0.30	85.71
Zinc	NA	0.15	+	NA	0.08	+	NA	0.41	+	0.96	0.43	44.80	0.8	0.40	50.0
Manganese	NA	0.06	+	0.12	0.119	99.16	NA	0.03 l	+	0.15	0.144	96.0	0.125	0.09	72.0
Iron	NA	0.41	+	0.6	0.76	126.66	1.0	0.4	40.0	0.15	0.12	80.0	0.125	0.09	72.0
Selenium	NA	ND	Nil	NA	0.0058	+	0.002	ND	Nil	NA	0.004	+	0.0008	ND	Nil
Chromium	NA	0.001	+	NA	0.002	+	0.002	0.005	250.0	NA	0.0003	+	NA	0.013	+
Vit. A (IU)	NA	ND	Nil	NA	ND	Nil	600000	498500	83.0	700000	674500	96.35	700000	604500	86.35
Vit. D3(IU)	NA	ND	Nil	NA	ND	Nil	120000	ND	Nil	70000	ND	Nil	70000	ND	Nil
Vit. E (IU)	NA	ND	Nil	NA	ND	Nil	150	ND	Nil	277	ND	Nil	277	ND	Nil

***Number of replicates, +Additional, wrt= with respect to**

Conclusion:-

In villages where there is lack of awareness regarding balanced rationing to fulfil the nutritional requirements of animals supplementation with good quality mineral mixture becomes more important and relevant. Study carried out at ARF R&D centre to evaluate the authenticity of label claims of the mineral mixture most commonly used in the area revealed the supply of non-area specific supplements and seven out of ten brands were found to be true to their label claims. Hence there is a pressing need to develop good quality mineral mixture to ensure the proper supplementation of deficient mineral mixture in the ration of dairy animals for better metabolism, maintaining good health, improved fertility, reduced duration between calving, hence optimal lactation. However, further study needs to be done on scientific basis so as to reach on final conclusion.

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