Explor. Anim. Med. Res., Vol. 1, Issue - 2, 2012. p- 161-166

IN VITRO EVALUATION OF A DIET SUPPLEMENTED WITH Mn ON NUTRIENT DIGESTIBILITY AND RUMEN FERMENTATION PATTERN IN CATTLE

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ABSTRACT : An experiment was conducted in 3 male rumen fistulated cattle fed on rice straw and concentrate based total diet to study the effects of supplementation of Manganese (Mn) from inorganic source (manganese sulphate) on in vitro dry matter (DM), organic matter (OM) & neutral detergent fibre (NDF) digestibility (%) & in vitro ammonia nitrogen (NH3-N) & total volatile fatty acid (VFA) production. These animals were offered total diet supplemented with 0 ppm Mn (T0) and 24 ppm Mn (T1) as per NRC, 2001 from inorganic source like manganese sulphate. After 21 days of feeding, rumen liquor samples were drawn at different time intervals (24 hour & 48 hour) to study the digestibility of DM, OM & NDF. For estimation of in vitro ammonia nitrogen (NH3-N) & total volatile fatty acid (TVFA) concentration each sample were incubated for 2 hour, 4 hour, 12 hour, 24 hour & 48 hour interval. This study inferred that supplementation of Mn through feed can improve the digestibility and rumen fermentation pattern and there will be more utilization of nutrient (crude protein, ether extract, organic matter). Trace mineral like Mn at per NRC (2001) recommendation was found more effective for the purpose than without supplementation in a diet.

Key words : Zn, Zinc sulphate, DM, OM, NDF, In vitro Digestibility, NH3-N , Total VFA production, Fistuted cattle .

INTRODUCTION:

For optimum production and maintenance of normal health of animals, it is important to provide essential nutrients in appropriate proportion including minerals. It is also essential to have a proper ratio of these minerals in the feeds and animal body because an improper ratio between minerals interferes the absorption of other and thereby resulting in deficiencies followed by low level of production and reproductive performance among ruminants (Suttle 1991). Though all the trace minerals are required to carry out the optimum functions of animal body but the micro minerals Mn, Cu, Zn and Fe are extremely important as they play vital physiological role in production and animal health (McDowell 2003).

Trace mineral like manganese (Mn) improves the growth of cellulolytic rumen microbes like *Ruminococcus spp*. and *Fibrobacter spp*. which result better fermentability and utilization of organic matter particularly hemicellulose (Tiwari *et al.*

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2000). Total diet with optimum supplementation of trace mineral combination provides an opportunity to manipulate the rumen eco-system for improving production, health status and overall performance by the animal. At the ruminal level, supplementation of trace minerals raise the amount of TCA- precipitable nitrogen indicating better utilization of NH3-N by rumen microbes which results in better organic matter and fibre digestibility. A significant increment ($P< 0.05$) can also be observed through total VFA production by the same treatment, which is indicative of greater utilization of non-structural polysaccharides at the ruminal level (Tiwari *et al.* 2000). In vitro study may assess the comparative effect of different feeds, fodders, minerals and enzymes added diet with respect to in vitro ammonical nitrogen and TVFA production at different hours of incubation and also estimate the in vitro DM digestibility (IVDMD), IVOMD, IVNDFD. The higher values of the above mentioned parameters are the indication of a direct correlation with superior type of feed that fulfills the actual requirement of macro and micro nutrients. In view of the above, the present experiment was conducted to study the effect of supplementation of Mn with a diet through in vitro nutrient digestibility & in vitro NH3-N & total VFA production in cattle on farm condition.

MATERIALS AND METHODS :

Three physically sound & normal cattle aged 3 year & weighing about 300 ± 50 kg were selected & fistulated at the left abdominal flank as per the standard surgical procedure (AFRC 1992) to collect rumen liquor for in vitro studies of basal diet with supplementation of inorganic mineral like Mn. A basal diet was formulated (Table 1) to meet the entire nutrient requirement according to NRC, 2001 except Mn. Chemical compositions of the diet were determined as per AOAC (1995), Goering and Vansoest (1970). Fistulated animals were fed basal diet with inorganic mineral Mn as 0 ppm and 24 ppm in 2 treatment groups like T0, T1 respectively (Table 2), twice daily each for 7 days interval with ad libitum clean drinking water.

The rumen liquor was collected on 8th day & 3 hour post feeding from different parts of rumen by suction. After collection into a pre warmed clean thermo-flask with temperature maintained at 39ºC, it was transferred to laboratory immediately & strained through 4 layers of muslin cloth to get Strained Rumen Liquor (SRL). About 0.5 gm sample from prepared substrate was taken in 100ml conical flask filled with a rubber cork with Bunsen valve. Then the sample was mixed with 40 ml of Mc Dougall's buffer & 10ml strained rumen liquor. In order to create anaerobic condition, CO2 gas was passed for 10 second through it & the stopper was put on the flask immediately. The conical flask was placed in B.O.D. incubator maintained at a constant temperature of 39ºC. The flask was shaken at regular intervals. Three replicates were taken for each hour of incubation. For determination of in vitro ammonia nitrogen (NH3-N) & total volatile fatty acid (TVFA) concentration each sample were incubated for 2 hour, 4 hour, 12 hour, 24 hour & 48 hour interval.

For estimation of in vitro dry matter digestibility (IVDMD), in vitro organic matter digestibility (IVOMD), in vitro neutral detergent fibre digestibility (IVNDFD) each flask were incubated for 24 hour & 48 hour separately. After the end of each incubation hour, the replicates were taken out. At the end of incubation, pH was measured immediately & the microbial activity of the flask was stopped by adding few drops of 1.07 (N) H2SO4 for TVFA & NH3-N estimation. The microbial activity of the flask was stopped with 1 ml of toluene for IVDMD estimation & was stored in a deep freeze for further analysis. The content of flask was centrifuged at 1500 rmp for 10 minutes & filtrate was used for the estimation of rumen parameter, NH3- N & TVFA. In vitro digestibility was determined according to the stage in vitro method developed by Tilley and Terry (1963),

Table 1 : Computation of total diet on DM basis (%)

Sl. No.	Ingredients	Basal Diet (%)
1	Rice bran	6.5
2	Wheat bran	10
3	Gram Chuni	10
4	Mustard cake	5
5	G.N.C.	2.5
6	Paddy straw	35
7	Hybrid Napier	25
8	Mollasses	5
9	Salt	1
	Total	100

Composition of experimental diet on DM basis (%)

DM	70.77
TDN*	56.82
OM	86.88
CP	11.74
CF	20.76
EE	2.87
NFE	51.51
NDF	53.50
ADF	37.54
TA	13.12
Mn, ppm	38.33

* Calculated value

Goering and Vansoest (1970). Similarly Ammonia nitrogen concentration in filtrate was estimated according to the procedure of Conway (1962) & TVFA concentration in SRL was estimated according to the procedure of Barnett and Reid (1957).

Statistical analysis for all the parameters under in vitro study were one way analysis of variance technique used for studying the main effect of two diets having one supplemented with Mn from inorganic sources and significant differences were calculated by post hoc test. From the experiment, the effect of supplementation of inorganic Mn in a basal diet was measured based upon their performances on in vitro DM, OM, and NDF digestibility.

RESULTS AND DISCUSSION:

The preparation & composition of experimental total diet and formulation of diet- mineral combination are presented in Table 1 and Table 2. The effects of In vitro digestibility (%) of dry matter (DM), organic matter (OM) and neutral detergent fibre (NDF) of basal diet supplemented with Mn at different hours of incubation have been presented in Table 3. Digestibility of DM, OM and NDF at both 24 and 48 hour incubation period increased significantly ($P<0.05$) in the treatment groups (T1) having supplementation of 24 ppm of Mn in diet. Besides, within each treatment groups, IVDMD (%), IVOMD (%) and IVNDFD (%) of substrates were more after 48 h rather than 24h of incubation period indicating 24h of incubation time is not sufficient for complete digestion of substrates by rumen microbes. Higher OMD in T1 probably due to presence of higher proportion of Mn in the substrate that resulted in better microbial activity resulting increased microbial fermentation leading to more production of TVFA leading to improved degradability and utilization of nutrients which may be further assessed through in vivo feeding trials in animals. The digestibility of NDFD (%) was affected by Mn supplementation which may be due to the fact that Mn requirement of the rumen microbes was not met from the basal diet.

So, treatment having Mn supplementation (24 ppm Mn) as per NRC (2001) performed better result on nutrient digestibility at two incubation period. The present findings are also in accordance with the ob-

Table 2: Formulation of different diet-mineral combinations

Treatment	Diet name	Type of Supplementation of trace mineral	Mn level (ppm)
Control(To)	Basal Diet	No supplement (Control)	0
T1		Mn from inorganic source as per NRC, 2001	24

servations reported by Tiwari *et al*. 2000 that intake of trace elements like Cu, Mn, Zn as 13.34, 7.62 and 125.73 (mg/day) respectively facilitate the growth of cellulolytic rumen microbes which causes better fermentability and utilization of OM, CP, cellulose and hemicelluloses. However, Arecovich *et al*. (2000) reported decreased DM digestibility linearly ($P<0.05$) as 44.7%, 44.3%, 42.8%, 43.2% and 40.9% by supplemental Zn at different level as 0, 5, 10, 15 and 20 ppm but addition of Mn at 0 ppm and 100 ppm increased ($P<0.2$) in vitro DM digestibility (44.7 vs 42.0) in heifer. But the present finding did not corroborate with the findings of Engle & Spears (2000) who reported that in vitro OM disappearance % and VFA were unaffected by supplementing graded level of Cu as 0, 10 and 20 ppm with fixed 20 ppm Mn and 30 ppm Zn in basal diet for steers.

Rumen fermentation pattern of NH3-N (mg/100 ml) and TVFA (meq/l) through supplementing trace mineral (Mn) in basal diet has been presented in Table 4 & 5. Statistical analysis revealed significant variation ($P<0.05$) for NH3-N and TVFA concentration between supplemented and non supplemented diet. The present findings of increasing trend of NH3-N (mg/100 ml SRL) and TVFA (Meq/litre SRL) concentration upto 4 hr of incubation followed by a decline at 12 hour and then again increasing trend to reach peak concentration at 48 hour. These trends were significantly varied between supplemented and non-supplemented diet. Considering all rumen parameters, it can be concluded that manganese (Mn) supplementation have improved diet quality. The macro and micro-elements have a substantial effect on the rumen microbial population (Hungate 1966). Supplemental minerals are directly involved with efficient utilization of diet and ultimately connected with production of animals (Singh *et al.* 2002).

Table 3: Effect of supplemental Mn with basal diet on *in vitro* digestibility (%)

Treatment	DMD 24h	DMD 48h	OMD 24h	OMD 48h	NDFD 24h	NDFD 48h
T0	54.86 ± 0.17^b	62.24 ± 0.17^b	56.76 ± 0.15^b	64.63 ± 0.10^b	40.40 ± 0.04^b	53.61 ± 0.03^b
T1	56.56 ± 0.24^a	63.79 ± 0.14^a	58.36 ± 0.29^a	65.54 ± 0.23^a	43.19 ± 0.30^a	56.32 ± 0.29^a

Besides significantly higher level of TVFA concentration and lower level of NH3-N by supplemental trace elements are the indicative of better utilization of NH3-N by rumen microbes for their own microbial synthesis indicating the positive response

Table 4 : Effect of supplemental Mn with basal diet on NH_3-N concentration (mg/100gm)

Treatment	VFA 2h	VFA 4h	VFA 12h	VFA 24h	VFA 48h
T0	107.68 ± 0.06^{b}	115.25 ± 0.07^{b}	96.34 ± 0.08^{b}	107.70 ± 0.09^{b}	126.58 ± 0.12^{b}
T1	108.73 ± 0.36^{a}	118.17 ± 0.25^{a}	97.43 ± 0.56^{b}	108.74 ± 0.58^{a}	129.16 ± 0.45^{a}

Table 5 : Effect of supplemental Mn with basal diet on TVFA concentration (meq/l)

Treatment	NH3-N 2h	NH3-N 4h	NH3-N 12h	NH3-N 24h	NH3-N 48h
T0	8.86 0.06^{b}	9.44 ± 0.06^{b}	7.13 ± 0.06^{b}	7.22 ± 0.01	9.12 ± 0.01^{b}
T1	9.22 ± 0.03^{a}	10.13 ± 0.07^{a}	7.55 ± 0.06^{a}	7.44 ± 0.06	10.06 ± 0.10^{a}

of trace mineral supplementation. The present findings support the observation of Bennink *et al.* (1998) with the view that levels of micro elements affected the rumen microorganism & its inside environment. Tiwari *et al.* (2000) observed significantly higher level of TVFA concentration and lower level of NH3-N concentration for diet supplemented with 13.34, 7.62 and 125.73 mg/day Cu, Mn and Zn for cows supporting the present findings in TVFA value for 50% excess mineral supplemented diet and in NH3-N value for NRC supplemented diet suggesting a better utilization of NH3-N by rumen microbes for their own microbial protein synthesis and better digestibility of OM, NFE due to higher level of TVFA value indicating an excellent response of trace mineral supplementation. Besides, higher value may associate with highly digestible soluble carbohydrate which generally favours propionate type of fermentation in rumen (Tiwari *et al*. 1998).

CONCLUSION:

It is evident from the study that supplementation of 24 ppm Mn in the basal diet improved the nutrient digestibility and higher rumen fermentation pattern like dry matter, organic matter and neutral detergent fibre, NH3-N and TVFA leading to more utilization of nutrients (crude protein, ether extract, organic matter) in the cattle. So, trace mineral like Mn at per NRC (2001) recommendation was found more effective for the purpose than without supplementation for preparation of ideal ration due to better nutrient utilization resulting better production and reproduction by further studies on in vivo experiment.

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