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Mineral Profile of Himalayan Pink and Black Salts and Their Efficacy on *in-vitro* Fermentation as Compared to Conventional Salt

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ABSTRACT

A study was undertaken to evalaute the mineral composition of pink and black Himalayan salts and their effect on *in- vitro* rumen fermentation. Three concentrate mixtures viz. CSCM (Conventional salt concentrate mixture); PSCM (Pink salt concentrate mixture); BSCM (Black salt concentrate mixture) were prepared by supplementing conventional common salt, Himalayan pink and black salts at 1% respectively. Concentrate mixture and maize silage were used in 60:40 ratio to prepare three different total mixed rations (TMR). Results revealed that sodium and chloride levels were 39.4 and 60.1; 27.8 and 42.8; 12.5 and 19.3 %, respectively in conventional, pink and black salts. Additionally, in pink and black salts, the level of iron, sulphur, magnesium and calcium were 1.10 and 5.38; 126 and 263; 66.2 and 0.88; 93.9 and 7.89 mg/100 g, respectively. Further, among heavy metal, the only Cd (0.1 ppm) was traced in the pink salt. *In-vitro* trials reported that there was no effect of replacing conventional salt with either pink or black on net gas production, *in-vitro* dry matter and organic matter digestibility, partitioning factor and microbial biomass production for substrate having either concentrate mixture or TMR. Therefore, it can be concluded that Himalayan pink and black salts could be beneficial in meeting the mineral requirement of dairy animals in economical way. However, a comprehensive *in- vivo* study is required to evaluate the effect of Himalayan rock salt (pink and black) on rumen fermentation and productive performance of dairy animals before drawing final recommendations.

KEYWORDS: Black himalayan salts, Dairy animals, Pink Himalayan salts, Minerals

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INTRODUCTION

Supplementation of sodium (Na) in the form of common salt/ table salt (Sodium Chloride; NaCl here onwards referred as conventional salt) to ruminants is a widely accepted practice and majority of prevalent feeding standards are recommending to add 1 % salt in the diet of ruminants throughout the world (Phillips et al., 1999). Na is an integral part of the animal body and is required for normal functioning of different physiological process like the regulation of extracellular fluid volume, nerve conduction, and muscle function. Chlorine (Cl) is one of the seven micronutrients essential for plants. Together with Na, it figures among those elements essential to living organisms including humans. In this way, sodium chloride (NaCl) present in salt becomes an important daily source of these elements (Pittarello et al., 2016).

Wild animals fulfil their requirement by licking different types of salt deposits/source in nature like stone/mountain/spring water etc, whereas the domesticated animals get it from the different feed ingredients and salt supplemented in their diet (Smith et al., 1953; Pittarello et al., 2016). Beyond white table salt, different variety of salts are available in the world like pulverized sea salt, commercial common salt (finely grounded), commercial salt blocks, Himalayan pink salt lump, Himalayan black salt lump etc. In recent years, Himalayan rock salts, particularly pink and black Himalayan salt, has gained more popularity throughout the world with its possible additional health benefits and is positioned to be nutritionally superior to white table salt (Fayet-Moore et al., 2020). Moreover, in India keeping lumps of Himalayan salt in mangers of dairy animals is a very common practice and it is practiced since ancient time. It is assumed to act as an appetiser and animals can lick these lumps as and when required to meet out their mineral requirement.

Few studies have reported the mineral content of pink salts internationally, and found pink salt to contain a variety of essential minerals like iron, zinc, and calcium, but found some samples also contained impurities or relatively large amounts of non-nutritive minerals such as arsenic, lead, and cadmium (Drake and Drake, 2011; Ul Hassan et al., 2016 and 2017; Chander et al., 2020; Fayet-Moore et al., 2020). On the other hand, very scanty scientific literature is available on impact of Himalayan salts on rumen fermentation of dairy animals either *in vivo* or *in vitro*. So, objective of present study is to assess major mineral profile along with mineral contaminants of Himalayan pink and black salt and to study the effect of these salts on *in-vitro* rumen fermentation when added in dairy animal's diet.

MATERIALS AND METHODS

Location

The study was carried out at the Department of Animal Nutrition, Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana (Latitude 30°542 62 2 N, Longitude 75°482 162 2 E, 240 m above sea level) in 2021-2022.

Sample collection, preparation and analysis

Lumps of pink and black colour salts (in duplicates) were collected, personally from the dairy farms of the villages Bahowal and Jallewal, district Hoshairpur, Punjab. Samples were crushed and grinded to powder form and labelled as Pink salt and Black salt and stored in air tight containers for further analysis.

Himalayan Pink Salt

Himalayan Black Salt

Substrate composition

Three different concentrate mixtures i.e., Conventional Salt Concentrate Mixture (CSCM); Pink Salt Concentrate Mixture (PSCM); Black Salt Concentrate Mixture (BSCM) were prepared by using conventional salt (sodium chloride), pink salt and black salt at 1 % level, respectively. Rest composition of all concentrate mixtures was same and comprised of maize 34 %, soybean meal 17 %, deoiled mutsrad cake 12 %, rice polish 10%, deoiled rice bran 24 % and mineral mixture 2%. These concentrate mixture and maize silage were used in 60:40 ratio to prepare three different Total Mixed Rations (TMR) viz. CSCM-TMR, PSCM-TMR and BSCM-TMR. The ingredient and chemical composition of the different concentrate mixtures and TMR is shown in Table 1.

Table 1. Ingredient composition and proximate analysis of concentrate mixture and total mixed ration (% DM) used during *in-vitro* experiment

Item	CSCM	PSCM	BSCM	CSCM-TMR	PSCM-TMR	BSCM-TMR
Maize	34	34	34			
Soyabean meal	17	17	17			
Deoiled mustard cake	12	12	12			
Rice polish	10	10	10			
Deoiled rice bran	24	24	24			
Mineral mixture	2	2	2			
Conventional Salt	1	-	-			
Pink salt	-	1	-			
Black salt	-	-	1			
Maize silage				60	60	60
CSCM				40	-	-
PSCM				-	40	-
BSCM				-	-	40
Proximate analysis						
OM	91.8	91.3	90.9	93.4	93.6	93.2
CP	22.6	21.9	22.5	15.8	15.8	14.9
EE	3.85	4.04	4.02	3.95	4.16	4.15
NDF	22.7	24.6	23.5	39.8	41.4	39.7
ADF	6.04	6.63	6.81	20.1	19.7	19.3
Hemi-cellulose	16.7	17.9	16.7	19.8	21.6	20.4
TA	8.19	8.67	9.12	6.54	6.35	6.8
AIA	1.91	1.91	1.86	2.4	2.26	2.4

CSCM: Conventional salt concentrate mixture; PSCM: Pink salt concentrate mixture; BSCM: Black salt concentrate mixture
OM: organic matter; CP: crude protein; EE: ether extract; NDF: Neutral detergent fiber; ADF: Acid detergent fiber; TA: total ash; AIA: Acid insoluble ash

In vitro evaluation

The effect of supplementing common salt, pink and black salt in concentrate and TMR (roughage: concentrate, 60:40) separately was carried out by *in vitro* gas production technique (Menke and Steingass, 1988). Rumen liquor was collected from fistulated animals maintained on conventional diet at the farm before feeding at 0900 h in a thermos flask flushed with CO_2 and maintained at 39ºC. The rumen contents were blended for 2-3 min in a blender and strained through four-layers of muslin cloth. The solution, containing 960 ml distilled water, 0.16 ml micro-mineral solution, 660 ml bicarbonate buffer,330 ml macro-mineral solution and 1.6 ml resazurin (0.1%) were mixed in a Woulff flask with magnetic stirrer in a water bath at 39ºC. The mixture was continuously flushed with CO_2. Then strained rumen liquor was added to the buffer media in the ratio of 1:2. Three samples were added to 100 ml calibrated glass syringes (Haberle Labortechnik, Germany) containing buffered rumen fluid. Blank and sample of standard hay were run in triplicate with each set. If the volume of gas in the syringe exceeded 70 ml after 8 h the volume was recorded and the gas was expelled. After 24 h, the volume of gas produced in each syringe was recorded and the contents of syringes were transferred to spout-less beaker. For estimation of *in-vitro* true dry matter digestibility (IVDMD), contents of spout-fewer beakers was refluxed with neutral detergent solution, filtered through sintered glass (G-1) crucibles and the residues were dried in hot air oven. The loss in weight was calculated and noted as IVDMD. *In-vitro* true organic matter digestibility (IVOMD) was determined by calculating loss of weight by ashing the remaining content in sintered glass (G-1) crucibles at 550°C for 2 h. The partitioning factor (PF) and microbial biomass production (MBP) were calculated from formula based on IVDOM (Blummel et al., 1997; Blummel et al.,1999).

Chemical and mineral analysis of samples

All three salt samples i.e., common, pink and black salt were sent for estimation of common major and heavy minerals by the inductively coupled argon plasma optical emission spectrophotometer at Interstellar Testing Centre Pvt.Ltd, Panchkula (Haryana). Further, samples of different concentrate mixture and TMR were processed for analysis of crude protein CP (# 984.13), ether extract (EE; # 920.39), neutral detergent fibre (NDF; # 2002.04), acid detergent fibre (ADF; # 973.18) and total ash (# 942.050) (AOAC, 2005). Hemi-cellulose was calculated as difference between NDF and ADF level in particular sample.

Statistical analysis

Data generated by *in-vitro* study were analysed using one-way analysis of variance (ANOVA) by SPSS, 2010 version 16 with model as follows:

$Y_{ij}=\mu+T_i+[_{ij}$, where, Y_{ij}=each observation (NGP; IVDMD; IVOMD etc.)

μ=overall mean, T_i=effect of ith treatment (i=Conventional salt, pink salt and black salt)

$[_{ij}$=residual error, Tukey's b test was used for the post-hoc comparison of different treatment means and statistical differences were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

The chemical composition of different concentrate mixtures and TMRs used as substrate in present study has been shown in Table 1. Different salts were added at 1% level in the concentrate mixtures and rest ingredients remained similar among various substrates hence, the content of CP, EE, NDF and ADF was found statistically similar. The major and heavy mineral composition of different salts is presented in table 2.

Table 2. Major minerals and heavy metal composition of various salts

Attributes	Conventional salt	Pink salt	Black salt
Na, % by mass	39.4	27.8	12.5
Cl, % by mass	60.1	42.8	19.3
Iron (mg/100g)	--	1.10	5.38
Sulphur (mg/100g)	--	126	263
Magnesium (mg/100g)	--	66.2	0.88
Calcium (mg/100g)	--	93.9	7.9
Cd, ppm	BLQ (LOQ 0.10)	0.10	BLQ (LOQ 0.10)
Pb, ppm	BLQ (LOQ 0.10)	BLQ (LOQ 0.10)	BLQ (LOQ 0.10)
As, ppm	BLQ (LOQ 0.10)	BLQ (LOQ 0.10)	BLQ (LOQ 0.10)
Hg, ppm	BLQ (LOQ 0.10)	BLQ (LOQ 0.10)	BLQ (LOQ 0.10)

Na: sodium; Cl: chloride; Cd: cadmium; Pb: lead; As: arsenic; Hg: mercury; BLQ: Below level of quantification; LOQ: Limit of quantification

Na and Cl were found to be 39.4 and 60.1; 27.8 and 42.8; 12.5 and 19.3%, respectively in conventional, pink and black salts. Conventional salt contains NaCl which has a 1:1 stoichiometry and it is made up by ^{23}Na (22.99 amu) and by ^{35}Cl (34.97 amu) and ^{37}Cl (36.97 amu) isotopes. Therefore, 100 g of sodium chloride has 39.34 g of Na and 60.66 g of Cl (Titler and Curry, 2011). Further, conventional salt was found to be devoid of any other major and heavy minerals. On the other hand, in pink and black salts, the level of Fe, S, Mg and Ca were 1.10 and 5.38; 126.08 and 263.12; 66.25 and 0.88; 93.97 and 7.89 mg/100 g, respectively. The S content was higher in black salt and other minerals (Fe, Mg and Ca) were found higher in pink salt and within the limits sets by WHO for salt in human consumption (5 g/kg for Ca and 3 g/kg Mg) as mentioned by Cheraghali et al. (2010). Further, among heavy metals the level of only Cd in the pink salt was found to be 0.1 ppm which is well below the maximum limits i.e., 0.2 ppm set by Codex. Other contaminants were found below the quantification level in both

Himalayan salts. These results are in agreement with previous reports of Sharif et al. (2007); Cheraghali et al. (2010) and Chander et al.(2020). Salts are usually white but inclusions of other minerals change its color and it is assumed that darker the salt, richer in the mineral composition. Moreover, the health beneficial effect against heartburn, bloating and flatulence of Himalayan salts for human beings is generally attributed to presence of various micro and macro elements particularly Fe (Duggal et al., 2015; Zuha et al., 2011). The macro element level is higher in black salt as compared to pink salt and due to higher level of S imparts typical sulphurous smell to black salt.

Results of *in vitro* trials reported that there was no effect of supplementing various types of salts in the concentrate mixture at 1 % on net gas production (NGP), *in-vitro* dry matter digestibility (IVDMD), in-*vitro* organic matter digestibility (IVOMD), partitioning factor (PF) and microbial biomass production (MBP) on substrate having either concentrate mixture or TMR (Table 3 and 4).

Table 3. Effect of supplementation of various salts on *in vitro* rumen fermentation characteristics of concentrate mixture

Attributes	CSCM	PSCM	BSCM	SEM	P value
NGP/g DM	189.0	188.2	187.3	4.35	0.930
IVDMD%	78.7	78.0	78.7	1.56	0.887
IVOMD%	83.0	84.6	85.0	1.78	0.935
PF	3.84	4.14	4.11	0.12	0.096
MBP (mg/mg OM digested)	60.1	71.0	69.7	3.74	0.052

CSCM: Conventional salt concentrate mixture; PSCM: Pink salt concentrate mixture; BSCM: Black salt concentrate mixture NGP: net gas production; IVDMD: *in vitro* dry matter digestibility; IVOMD: *in vitro* organic matter digestibility; PF: partitioning factor; MBP: microbial biomass production

Table 4. Effect of various salts on *in vitro* rumen fermentation characteristics of total mixed ration

Attributes	CSCM-TMR	PSCM-TMR	BSCM-TMR	SEM	P value
NGP/g DM	208.2	213.2	216.5	4.19	0.216
IVDMD%	67.0	67.7	67.0	1.01	0.758
IVOMD%	71.9	72.9	72.6	1.06	0.669
PF	3.14	3.15	3.19	0.072	0.767
MBP (mg/mg OM digested)	37.1	38.8	40.1	2.51	0.531

CSCM-TMR: Conventional salt concentrate mixture based total mixed ration; PSCM: Pink salt concentrate mixture based total mixed ration; BSCM: Black salt concentrate mixture based total mixed ration. NGP: net gas production; IVDMD: *in vitro* dry matter digestibility; IVOMD: *in vitro* organic matter digestibility; PF: partitioning factor; MBP: microbial biomass production

Critical pursual of data revealed that when concentrate mixture-based substrate was used, there was a tendency (P=0.052) of higher MBP with pink and black salt supplementation as compared to conventional salt in both *in vitro* studies. Though the values in TMR based substrate was also numerically higher in pink and black salt but remained statistically non-significant. For maintaining normal rumen microorganism functioning the diet of animals not only balanced for carbohydrate, protein and fat level but micronutrients also need special attention. In previous *in vitro* studies excessive amount of trace minerals in the diet of animals had negative effect on rumen microbial populations and fermentation (Durand and Kawashima, 1980; Durand and Komisarczuk, 1988; Arelovich et al., 2000;).

Therefore, the result of present study revealed that, there was no negative effect of either pink or black salt on rumen fermentation characteristics. On the other hand, deficiency of minerals has negative effect on rumen fermentation characteristics which may impact reproductive and productive performance of dairy animals (Martinez and Church, 1970; Preston and Lang, 1987).

India holds first rank in milk production and as per latest 20th livestock census, the number of milch animals (cows and buffaloes) is increased by only 6 % since the 2012 (19th livestock census) whereas milk production increased by 58.5 % during this period. These data revealed that rising productivity rather than expansion in herd size is the principal engine behind the rise in India's milk production. So, apart from balancing ration for energy and protein, mineral nutrition also needs a special attention. As in India, in unorganized dairy sector and at household level dairy animals are generally fed on crop residues, natural grasses, tree leaves and shrubs in which minerals are either low in concentration or their bioavailability to host animals is not much known (Prasad and Gowda 2005).

CONCLUSIONS

From present study it can be concluded that Himalayan pink and black salts are not only good source of Na and Cl but also of S, Mg, Ca and Fe. Moreover, the level of heavy metal contaminants was found well below the recommended level by codex inedible salts. Further, supplementation of pink and black Himalayan salts at 1% replacing conventional salt has no negative effect on rumen fermentation characteristics of substrate having either concentrate mixture or TMR. Therefore, replacing conventional salts by Himalayan pink and black salts could be beneficial in meeting some of the mineral requirement of dairy animals in economical way. However, a comprehensive *in-vivo* study is required to evaluate the effect of Himalayan rock salt (pink and black) on rumen fermentation and productive performance of dairy animals before drawing final recommendations.

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