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ORIGINAL ARTICLE

Comparative milk metabolite profiling for exploring superiority of indigenous Indian cow milk over exotic and crossbred counterparts

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Abstract This study was planned to identify differences in the milk metabolite composition of Indian (Sahiwal), exotic (Holstein–Friesian) and their crossbred cows in intensive system of management. To mimic the management system of ancient India, indigenous cattle under extensive system (zero input) were also included. Holstein–Friesian (HF) had significantly higher amount of saturated fatty acids (SFA, 76.3%) as compared to the crossbred (73.3%) and Sahiwal (68.0%). HF had the highest concentration (42.7%) of hypercholesterolemic fatty acids and the maximum value (68.5) of athrogenecity index (AI). Sahiwal had the highest proportion (32.1%) of total unsaturated fatty acids (UFA). Mineral, vitamin, n-3 fatty acids and total amount of essential amino acids did not vary across the three groups. Milk of indigenous cattle maintained only on grazing had more favorable nutrient profile. It had low SFA (61.4%), high UFA (38.6%) and higher concentrations of both monounsaturated fatty acids (31.4%) and polyunsaturated fatty acids (7.2%). The n-6/n-3 ratio (2.7) and the AI (33.9) were significantly lower. Significantly higher concentrations of minerals (Zn, Fe, P and Cu) and vitamins except vitamin B5 were recorded in their milk. The study revealed that milk metabolite characteristics can be used to promote indigenous cattle.

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Introduction

The bond between cattle and humans is continuing since thousands of years as cattle provide milk, meat, manure and draught power to mankind. Cattle (*Bos taurus*) were domesticated around 8500 years BC in the Near East from their wild progenitor (*Bos primigenius*). Widespread modern zebu (*Bos indicus*), or humped cattle was domesticated from its predecessor, the aurochs (*Bos primigenius namadicus*) at about 6000 years BC in the Indus Valley (Bollongino et al. 2012). Distribution of cattle in different regions led to the development of several ecotypes which subsequently became the present day breeds. India's rich cattle bio-diversity is reflected in 41 well-defined breeds (www.nbagr.res.in). Majority of these are draught breeds, some are dual purpose breeds, for instance Kankrej, Hariana and Ongole, and only five (Tharparkar, Sahiwal, Red Sindhi, Gir and Rathi) are recognized for their milking proficiency. Crossbreeding program with high milk producing *Bos taurus* cattle was initiated in India nearly a century ago to gain a boost in the milk production. These efforts transformed India from a milk deprived nation to the largest milk producer (163.7 million tonnes in 2016–2017) in the world (DAHD 2017). At present, India has 190.90 million cattle of which 151.17 million are indigenous cattle and 39.73 million are exotic/crossbred cattle (Livestock census 2012).

Ayurveda, the Indian system of medicine, has described numerous benefits of Indian cow milk (Burjor 2007). Cattle farmers throughout the country proclaim the benefits of indigenous cow milk over the milk of high-yielding exotic

breeds for physical and mental growth of children. The superiority claimed for Indian cow milk might have association with the management practices that were followed during ancient times. *Rig Veda Samhita*, the oldest extant Indic text (2nd millennium BC) does mention (*Rig Veda Mantra* 10-169-1 and RV10-1692) about such feeding practices of the cows (Burjor 2007).

Milk composition does tend to differ among countries due to use of different breeds and feeding practices and different breeding policies. Several studies have reported comparative milk composition of their native cattle and commonly used breeds across the world including their crossbreds (Samkova et al. 2012). Milk composition of cows under extensive system of management has also been profiled because their milk is obtained without adversely affecting environment and is perceived to be a natural food with nutritional and health benefits. There is a dearth of knowledge in this regard from Asia which is reflected in recently published meta-analyses of organic and conventional bovine milk (Srednicka-Tober et al. 2016). Majority of publications are from Europe (76%), with some remainder originating mainly from Brazil and the USA.

The experiments conducted for estimating the effect of genetic variation, physiological factors or feed modifications on milk production have indirectly provided some insights into composition of milk of Indian cattle. However, these studies were conducted on different farms, under diverse environmental and management conditions. Controlled investigations on differences between *indicine and taurine* milk composition have so far been mostly evasive. Dairying is not a large scale enterprise in India and very few government and institutional farms maintain indigenous, exotic and cross-bred cattle at one place, thus limiting the scope for any scientific study. Information on the composition of milk derived from Indian cattle traditionally being maintained entirely on the zero input system does not exist at all.

Therefore, the principal objective of this study was to generate comprehensive milk metabolite profile of Indian, exotic and crossbred cows, maintained at one livestock farm, under similar production system and using identical methodologies. In addition, milk composition of indigenous cattle raised in extensive system of management was also analyzed with a view to provide scientific data for the superiority of indigenous cow milk, if any. This publication presents the most comprehensive compilation of the milk metabolite composition of Indian cattle both in terms of management system (intrinsic and extrinsic) and nutrient component, analyzed till date.

Materials and methods

Selection of animals and management conditions

Indigenous (Sahiwal), exotic (Holstein–Friesian) and their crossbred (50% exotic) cows (ten each) were selected from Government Livestock Farm, Hisar. This farm is maintained by Department of Animal Husbandry and Dairying, Government of Haryana, India. The farm is located in north India, at latitude 29°10′ north and longitude 75°43′ east, at 214 m above sea level. The area has tropical monsoon climate with 450 mm of average annual rainfall. Annual average maximum and minimum temperatures are 31.5 and 16.2 °C, respectively. Cows were kept under intensive management conditions at farm. Care was taken to minimize the effect of variables affecting milk composition (season, parity, lactation stage, feed and fodder and health condition) while selecting the animals. Healthy animals of first parity in the middle stage of lactation (110–130 days) were selected.

All the cows (n = 10) under extrinsic system of management belonged to indigenous cattle (*Bos indicus*) and were owned by farmers. These animals were maintained in an open housing system. It was built mostly from local resources in the vicinity of owner's house. Supplementary food was uncommon. Animals were let loose on grazing daily from morning to evening. Hand milking of the cows was carried out before and after grazing. The effect of common variables (nutrition and environmental factors) on milk composition was minimized by keeping the study area small that was having the same vegetation. There was no prior knowledge about the species and nutritional quality of the plants on which these animal graze due to spatial heterogeneity of palatable vegetation resources.

Milk sample collection

Collection of milk samples was carried out during the month of March, 2016. Forty milk samples were collected which included thirty from the farm (Sahiwal-10, Holstein–Friesian-10 and crossbred-10) and ten from the field (indigenous cattle maintained under zero input system). Milk sample collection was done simultaneously for all the three cattle groups on the farm. Morning milk samples were collected for three successive days. Hand-milking without the use of any milk-letting agent was followed. Milk of all the four quarters was pooled. Milk samples were transferred to 500 mL sterile plastic bottles and were stored in a deep freezer at − 20 °C. The samples were transported to the laboratory for analysis in a cold box. Milk collected from indigenous cattle under extensive system of management was stored on ice for approximately

1–2 h until freezing services were accessible. Samples were transported in frozen state to the food analysis laboratory within 24 h for metabolite analysis.

Chemical analysis

Milk sample analysis was carried out by Punjab Biotechnology Incubator, Mohali (India). This food testing laboratory is accredited by the Bureau of Indian Standards (BIS) and National Accreditation Board for Testing and Calibration of Laboratories (NABL). Analytical grade reagents, high purity solvents for chromatographic technique, and deionized (Milli-Q system, Millipore, Bedford, MA, USA) water were used. All reagents, solvents and standards were purchased from Merck (Darmstadt, Germany), Supelco Inc (Bellefonte, PA, USA) and Sigma Aldrich (St Louis, MO, USA). In addition, authentic reference samples were taken from in-house collection of food testing laboratory.

Analytical methods

Samples belonging to one cow were pooled before analysis. The proximate parameters were analyzed by using AOAC (2010). Analyses of fatty acids were carried out by the method of Ranganna (1986). Fatty acid composition was established using gas liquid chromatodraphy (GLC) fitted with HP-88 column and flame ionization detector (Varian 3800CP, CA, USA). The data generated on the fatty acid composition of milk was further analyzed to determine content of total saturated fatty acids (SFA), total unsaturated fatty acids (UFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). Additionally, following indices were calculated: Δ^9-desaturase (Samkova et al. 2012) as product of Δ^9-desaturase/(product of Δ^9-desaturase + substrate of Δ^9-desaturase) and the atherogenecity index (AI) as per the method of Chillard et al. (2003), thus (C12:0 + 4x C14:0 + C16:0)/(MUFA + PUFA). Cholesterol was estimated by AOAC 994.10 method (AOAC 2010) using DB-5ms column with GC-FID (Varian 3800CP).

Minerals (Na, K, Ca, Mg, Fe, Cu and Zn) were quantified using inductively coupled plasma mass spectrometry (ICP-MS) and Phosphorus by the iCAP-AES system (Thermo electron, 6300) as per AOAC 999.10 official method (AOAC 2010). Nitric acid and hydrogen peroxide were used for digestion of samples to eliminate organic matter. Sample was injected on ICP-MS (Agilent-7700x) by using helium collision mode for determination of minerals. Vitamin A was estimated as per AOAC method 2001.13 (AOAC 2010) using HPLC (Agilent Technologies, 1200 Series, CA, USA) on column ZorbaxSil equipped with diode array detector (DAD) (G1315D, Agilent Technologies). Quantification of vitamin E was as per AOAC method 971.30 (AOAC 2010) using the same column and HPLC equipped with Fluorescence detector (FLD: G1321A, Agilent Technologies, CA, USA). Vitamin C was analyzed using the titrimetric method as per AOAC method 967.21 (AOAC 2010). Vitamin B1, B2, B3 and B6 were analyzed as per British Standards Institution (2014) methods using HPLC (Agilent Technologies, 1200 series) with FLD (G1321A, Agilent Technologies) detector and Eclipse XDB C18 column. β-carotene was estimated as per the method of Ahamad et al. (2007).

Amino acid composition was determined by using standard method prescribed by Waters® AccQ-Tag™ (Waters Corporation, Milford, MA, USA). AccQ-Fluor Reagent Kit (WAT052880) was used for the sample preparation.

Statistical analysis

All data were analyzed by the SPSS 10.0 software package (SPSS Inc., Chicago, Illinois, USA) and presented as mean of the triplicate analyses. The mean values of the different groups were compared by one-way ANOVA followed by Post Hoc Test at 95% confidence level ($P < 0.05$).

Results and discussion

Sahiwal was selected to be the representative of Indian cattle being the best milch breed in the tropics including India. Selection of Holstein–Friesian (HF) and HF crossbred were based on the preference for these animals in the current crossbreeding scenario of India (Singh 2016). It has been established that exotic inheritance beyond 50% does not result in appreciable gain (Singh 2016). Thus, Sahiwal, HF and their crossbred (50% exotic inheritance) were selected for the comparison.

Indian cattle traditionally being maintained completely under zero input system might be considered closest to the cattle described for their positive attributes in Indian scriptures. These cattle used to roam freely in the fields and forests and were not provided with supplementary feed. Moreover, they have been reared traditionally without any effort to improve them for commercial milk production.

Proximate composition

Table 1 presents the proximate composition of milk obtained from three groups of cows at farm (intensive management) and indigenous cattle under extrinsic system of management.

Table 1 Milk proximate composition (%) of Sahiwal, Holstein–Friesian and their crossbred cows at farm and of indigenous cattle under zero input system in the field

	Sahiwal		Holstein–Friesian		Crossbred		Indigenous cattle		Level of significance
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	
Total solids	13.557[a]	0.168	12.618[b]	0.298	12.623[b]	0.600	14.360[a]	0.184	**
Protein	3.027[a]	0.079	2.790[a]	0.056	3.088[a]	0.142	3.371[b]	0.103	**
Fat	4.012[a]	0.066	3.400[b]	0.033	3.600[b]	0.103	4.989[c]	0.164	***
Ash	0.718	0.009	0.730	0.017	0.718	0.010	0.743	0.013	NS
Lactose	5.413[a]	0.063	5.138[b]	0.062	5.318[ab]	0.066	5.391[a]	0.078	*

Each value represents the mean of observations on ten animals

NS non significant

Mean with different superscript(s) in a row differs significantly

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

Intensive system of management

There was no significant difference ($P < 0.05$) in the ash content among the compared cattle groups. No difference was recorded in the proximate composition of HF and crossbred cattle. Percentage of fat, total solids and lactose was significantly enhanced in the milk of Sahiwal as compared to that of HF. Protein content did not vary between the two (Table 1). Proximate composition was in agreement with the data reported in the literature for *Bos taurus* and *Bos indicus* cattle. Fat and total solid percentage for Indian dairy cattle have been reported to vary between 3.5–5.5 and 12.20–15, respectively (Banerjee 2009). Percentage of fat and total solids varied between 3.41–5.06 and 12.27–14.54, respectively in five (Brown Swiss, Ayrshire, Holstein, Guersey and Jersey) exotic breeds (Nevens 2010). Schonfeldt et al. (2012) reported milk lactose concentration from six countries of which Denmark had the lowest (4.64 g/100 g) and USA had the highest values (5.26 g/100 g). Milk protein concentration was considered to be unaffected by differences in nutrition and management. Largest differences from various countries were observed in the total fat content. Fat content of milk from USA was reported to be 3.25 g/100 g, while it was 4.0 g/100 g in the databases of Australia and New Zealand. Breed differences were also reported as Holstein had the lowest fat and protein content, while Jersey and Guernsey had the highest (Schonfeldt et al. 2012).

In the current study, higher concentration of lactose (Table 1) was recorded in the milk of Indian cattle (5.4%) as compared to the HF (5.1%). These findings are in line with the results (5.26%) reported for Bangladesh cattle (*Bos indicus*) having higher concentration of lactose as compared (4.59%) to the Holstein crossbred (Islam et al. 2014). Unlike the present result, breed differences were not significant with respect to lactose among Holstein, Brown Swiss, Jersey, and Ayrshire (Bleck et al. 2009). It is worth mentioning that aforesaid breeds belong to *Bos taurus* whereas, differences recorded in the current study were between *Bos indicus* (Sahiwal) and *Bos taurus* (HF).

Extensive system of management

Grazing indigenous cattle produced milk richer in protein and fat (Table 1). A meta-analysis of difference in the nutritional value between dairy products corresponding to conventional and organic category concluded higher fat and protein in organic milk (Srednicka-Tober et al. 2016). Significantly higher protein, fat and total solids in the milk of Red Chittagong, an indigenous cattle (*Bos indicus*) of neighboring country, Bangladesh as compared to that of crossbred (Jersey, Holstein and Sahiwal) has been recorded by Islam et al. (2008). These observations coincide with the current findings of higher concentration of protein (3.37 ± 0.1) in the milk of indigenous cattle maintained on grazing (Table 1). However, protein percentage in the milk of Indian native cattle under extensive system (3.37%) was lower than that of cows maintained under traditional pastoral system in Nigeria (3.54–3.68%) (Adesina 2012) and Bangladesh (4.06%) (Islam et al. 2008). System of management (intensive or extensive) did not affect the lactose content (Table 1) in accordance with the number of publications reporting similarity in content of lactose, irrespective of organic or conventional milk production.

Fatty acid profile

In the current study, 50 traits were studied which include, 31 individual fatty acids (saturated-16, monounsaturated-7 and polyunsaturated- 8), 10 groups of fatty acids and 8 indices and cholesterol. The results are presented in Table 2 as the percentage of total fatty acids. The profile of

Table 2 Milk cholesterol concentration and fatty acid composition (fatty acids, group of fatty acids, ratios and indices) from Sahiwal, Holstein–Friesian and crossbred cows maintained in intensive and indigenous cattle in extensive system of management

	Sahiwal		Holstein–Friesian		Crossbred		Indigenous cattle		Level of significance
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	
Cholesterol, (mg/100 g milk)	10.69	0.872	11.77	0.670	10.15	0.821	12.33	0.187	NS
Fatty acids (g/100 g of total fatty acids)									
Saturated fatty acids									
C4:0	0.034^{a}	0.014	0.214^{b}	0.030	0.099^{c}	0.024	0.020^{a}	0.002	***
C6:0	0.566^{a}	0.097	2.563^{b}	0.129	1.802^{c}	0.267	1.066^{d}	0.108	***
C8:0	3.047^{a}	0.204	1.843^{b}	0.098	1.519^{bc}	0.114	2.155^{bd}	0.165	***
C10:0	6.525^{a}	0.223	4.214^{b}	0.304	3.357^{c}	0.179	3.689^{bc}	0.200	***
C11:0	0.348^{a}	0.029	0.293^{ab}	0.030	0.248^{b}	0.011	0.038^{c}	0.009	***
C12:0	4.926^{a}	0.256	4.590^{ab}	0.212	4.151^{bc}	0.167	3.831^{c}	0.152	**
C13:0	0.274^{a}	0.027	0.149^{b}	0.010	0.116^{b}	0.013	0.152^{b}	0.014	***
C14:0	9.967^{a}	0.099	15.744^{b}	0.613	14.171^{c}	0.599	7.393^{d}	0.172	***
C15:0	4.603^{a}	0.224	2.688^{b}	0.248	3.335^{b}	0.130	2.591^{b}	0.154	***
C16:0	19.651^{a}	0.095	22.371^{b}	0.820	22.384^{b}	0.416	19.355^{b}	0.394	***
C17:0	1.288^{a}	0.125	1.134^{a}	0.063	1.310^{a}	0.092	2.412^{b}	0.130	***
C18:0	15.574^{a}	0.649	19.422^{b}	0.410	19.978^{b}	0.563	17.426^{c}	0.113	***
C21:0	0.324	0.033	0.519	0.044	0.406	0.041	0.494	0.086	NS
C22:0	0.487^{a}	0.074	0.247^{b}	0.027	0.266^{b}	0.017	0.573^{a}	0.067	***
C23:0	0.129	0.032	0.076	0.028	0.052	0.005	0.063	0.014	NS
C24:0	0.218	0.069	0.202	0.068	0.144	0.011	0.181	0.014	NS
Monounsaturated fatty acids (MUFA)									
C14:1	5.067^{a}	0.162	3.309^{b}	0.117	3.045^{b}	0.127	3.347^{b}	0.267	***
C16:1	6.299^{a}	0.663	3.055^{b}	0.157	2.561^{b}	0.108	3.140^{b}	0.039	***
C17:1	1.070^{a}	0.163	0.422^{b}	0.036	0.575^{b}	0.040	1.165^{a}	0.088	***
C18:1n-9	12.852^{a}	0.328	11.181^{b}	0.379	14.565^{c}	0.346	21.633^{d}	0.521	***
C20:1n-9	1.626^{a}	0.062	0.761^{b}	0.105	1.178^{c}	0.129	1.523^{acd}	0.178	**
C22:1n-9	0.076^{a}	0.017	0.035^{a}	0.003	0.052^{a}	0.009	0.535^{b}	0.091	***
C24:1n-9	0.113^{a}	0.017	0.059^{b}	0.009	0.044^{b}	0.007	0.061^{b}	0.011	**
Polyunsaturated fatty acids (PUFA)									
C18:2n-6 (LA)	2.734^{a}	0.129	2.515^{ab}	0.071	2.398^{b}	0.093	3.930^{c}	0.127	***
C18:3n-6	0.672	0.047	0.597	0.022	0.617	0.015	0.625	0.060	NS
C18:3n-3 (ALA)	0.873^{a}	0.040	0.730^{a}	0.042	0.782^{a}	0.031	1.595^{b}	0.069	***
C20:2n-6	0.060^{a}	0.004	0.390^{bc}	0.065	0.208^{ab}	0.074	0.585^{c}	0.112	**
C20:3n-6	0.088	0.029	0.210	0.052	0.101	0.008	0.112	0.022	NS
C22:2	0.016^{a}	0.000	ND	ND	ND	ND	0.042^{a}	0.004	
C20:5n-3 (EPA)	0.088^{a}	0.021	0.041^{ab}	0.006	0.033^{b}	0.004	0.150^{c}	0.023	***
C20:4n-6 (AA)	0.469^{a}	0.072	0.478^{a}	0.042	0.381^{a}	0.018	0.143^{b}	0.007	***
Total SFA	67.958^{a}	0.310	72.268^{b}	0.299	73.337^{c}	0.367	61.442^{d}	0.681	***
Groups									
Short chain (C4-C8)	3.646^{a}	0.190	4.620^{b}	0.187	3.420^{a}	0.389	3.240^{a}	0.273	**
Medium chain (C10-C14)	22.039^{a}	0.425	24.989^{b}	0.743	22.042^{a}	0.473	15.104^{c}	0.509	***
Long chain (> C16)	37.670^{a}	0.497	43.971^{b}	1.147	44.540^{b}	0.904	40.505^{c}	0.374	***
Hypercholesterolemic fatty acids (HFA)	34.544^{a}	0.271	42.705^{b}	0.848	40.705^{c}	0.635	30.579^{d}	0.519	***
Total MUFA	27.102^{a}	0.422	18.820^{b}	0.212	22.019^{c}	0.287	31.404^{d}	0.441	***
Total PUFA	4.998^{a}	0.172	4.880^{a}	0.113	4.520^{a}	0.206	7.182^{b}	0.218	***
Total Unsaturated FA (UFA)	32.100^{a}	0.336	23.700^{b}	0.202	26.539^{c}	0.454	38.586^{d}	0.558	***
Total n-6 fatty acids	4.022^{a}	0.243	4.190^{a}	0.092	3.705^{a}	0.120	5.395^{b}	0.148	***

Table 2 continued

	Sahiwal		Holstein–Friesian		Crossbred		Indigenous cattle		Level of significance
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	
Total n-3 fatty acids	0.960[a]	0.042	0.770[b]	0.043	0.815[ab]	0.032	1.745[c]	0.069	***
Ratios									
LA/ALA	3.133[a]	0.262	3.446[a]	0.196	3.065[a]	0.093	2.464[b]	0.147	**
n-6/n-3 ratio	4.188[a]	0.418	5.441[b]	0.345	4.546[a]	0.128	3.091[c]	0.164	***
H/U (HFA/UFA)	1.077[a]	0.016	1.802[b]	0.036	1.538[c]	0.046	0.794[d]	0.023	***
S/U (SFA/UFA)	2.117[a]	0.031	3.218[b]	0.036	2.763[c]	0.057	1.592[d]	0.038	***
Indices									
C14:1 Δ^9-desaturase	0.337[a]	0.006	0.174[b]	0.006	0.178[b]	0.009	0.310[a]	0.018	***
C16:1 Δ^9-desaturase	0.240[a]	0.019	0.121[bc]	0.008	0.103[b]	0.005	0.140[c]	0.002	***
C18:1 Δ^9-desaturase	0.453[a]	0.007	0.365[b]	0.009	0.422[c]	0.006	0.553[d]	0.007	***
Athrogenicity index	45.406[a]	0.642	68.510[b]	2.483	61.681[c]	2.341	33.906[d]	0.815	***

Each value represents the mean of observations on ten animals

ND not detectable, *NS* non significant

Mean with different superscript(s) in a row differs significantly

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

cows' milk FA from exotic (*Bos taurus*) dairy breeds is well documented (Srednicka-Tober et al. 2016), however a comprehensive comparison with the published literature was not made. The primary reason for this is high variability in milk FA composition among breeds across studies (Eijndhoven et al. 2011) because of inherent (species, breed, genotype, pregnancy, stage of lactation) and external (feed and environment) factors (Myburgh et al. 2012; Islam et al. 2014). Detailed report on the milk FA of the *indicine* cattle could not be found in the published literature. There have been few studies on indigenous cows which mainly focused on the variation in fatty acid profile due to the difference in the dietary regimens, involved different breeds and were conducted on different farms having their own set of management conditions (Tyagi et al. 2010).

Intensive system of management

Cholesterol content was not different across the cattle groups (Table 2). Its concentration was within the range (10–14 mg/100 g) reported for whole bovine milk (Schonfeldt et al. 2012). Significant differences ($P < 0.05$) were recorded in total SFA and UFA across the groups. Majority of individual SFA differed significantly between Sahiwal, HF and crossbred cattle. HF cows produced significantly higher proportion of SFA (76.3%) as compared to crossbred (73.3%) and Sahiwal (68.0%). This difference was due to the higher percentage of all the three groups of SFA (short, medium and long chain) in the HF milk as compared to Sahiwal (Table 2), whereas, the difference in the SFA content between Sahiwal and crossbred was only due to the variation in long chain fatty acids (> C16:0). The SFA scenario was just opposite between HF and crossbred as short and medium chain fatty acids varied significantly. In the SFA category, Palmitic acid (C16:0) had the highest proportion followed by Stearic (C18:0) and Myristic acid (C14:0) across all the groups. However, concentration of Myristic acid (C14:0) was substantially higher in the HF and crossbred cattle (Table 2). Total C4:0–C8:0 FA was observed to be lower in all the cattle groups. Devle et al. (2012) also reported a lesser amount of short chain FA in Norwegian cow. Higher proportion of SFA in the milk of HF cattle corresponded with the observations of Kay et al. (2005), that the selection of animals for higher milk yield resulted in increased proportion of SFA.

The trend observed in total UFA was just the opposite of SFA (Table 2). Sahiwal had the highest value (32.1%) followed by crossbred (26.5%) and HF (23.7%). This difference was due to the significantly different MUFA levels. The total PUFA level was similar in the milk of Sahiwal, HF and crossbred cattle. Individual PUFA, including α-linolenic acid (ALA) was at par in the milk of HF and Sahiwal. Identical feeding on the farm can be the reason for this observation as ALA is primarily of dietary origin. Total n-6 FA did not show any variation among *indicine*, *taurine* and crossbreds at the farm (Table 2). Lower percentage of total n-3 FA was observed in the HF milk (0.77%) as compared to Sahiwal (0.96%). It was not due to ALA, the major n-3 FA, but was because of significantly lower Eicosapentaeoic acid (EPA). Milk of crossbred cattle

did not differ significantly, either from Sahiwal or HF in total n-3 FA.

Extensive system of management

The SFA proportion was lower (61.40%) in the milk of indigenous cattle under extensive system of management (Table 2). This decrease was mainly attributed to the medium chain FA group (15.1%). It was even less than the least value observed among the farm animals (Sahiwal, 22%). This milk can be considered as an augmented source of UFA (38.6%). Both MUFA (31.4%) and PUFA (7.2%) were significantly higher than the farm managed HF and crossbred cows. Among MUFA, oleic acid (21.6%) was the main driver of increased MUFA level. Majority of individual PUFA had greater concentrations (Table 2). Value recorded for ALA, was almost twice as compared to that recorded in intensive system of management. As a result, the milk was enriched in total n-3 fatty acids. Similarly, linoleic acid (LA), the chief n-6 fatty acid as well as the total n-6 fatty acids were significantly elevated in the milk of grazing cattle. Enhanced levels of ALA and EPA in the milk of cows grazing on pastures than the cows feeding on conventional diets have been reported from different countries (Schwendel et al. 2015). Very long chain fatty acids and corresponding unsaturated derivatives (> C20) were also detected in grazing cattle milk, although their concentrations were very low (Table 2). Myburgh et al. (2012) also reported similar observations in the African cattle breeds maintained under free ranging system.

Dissimilarity in the FA profile of indigenous cows maintained under farm and those in the zero input system may very well be attributed to the feeding practices (Table 2). Diet of the cows kept on the farm did not contain grazed grass, whereas, cows under extensive system were totally on forest grazing. Eijndhoven et al. (2011) reported that grazing or nongrazing based feeding immensely influenced milk FA composition. Milk FA composition was also influenced by the quantity and quality of available forages. Different FA profile of milk from indigenous cattle thriving on grazing may also be due to the unique symbiosis with specific rumen bacteria which prefer different FA pathways.

Fatty acid indices

Greater attention is paid nowadays to the proportion of n-3 and n-6 PUFA in the diet. Higher concentration of n-6 FA is associated with possible negative health issues (e.g., cancer, heart, autoimmune and inflammatory diseases). A low ratio of n-6 to n-3 FA is considered to be beneficial for human health (Schwendel et al. 2015). Milk fat is by and large considered to be proatherogenic due to the SFA content and more precisely due to the larger proportion of hypercholesterolemic fatty acids (HFA) namely lauric, myristic and palmitic. The atherogenic index (AI) is considered as a criterion for atherogenic properties of milk being based on level and interaction of fatty acids contributing towards this condition (Srednicka-Tober et al. 2016).

Intensive system of management

The ratio of n-6/n-3 was significantly higher in the HF (Table 2). The observed n-6/n-3 ratio in the Sahiwal milk (4.2) was very close to the ideal values suggested for human diet (2:1 to 4:1; Sretenovic et al. 2009). Steroyl-CoA desaturase (Δ^9-desaturase) is the prime enzyme in FA metabolism which regulates the desaturation of SFAs to its corresponding MUFA. The Δ^9-desaturase indices were significantly lower for the HF and crossbred cattle as compared to Sahiwal (Table 2). Lower Δ^9-desaturase indices corresponded to the reduced fraction of MUFA in the milk (Table 2). Lowest value of HFA and AI were recorded for Sahiwal followed by crossbred and highest for HF, amongst the farm animals (Table 2). Lower value of Δ^9-desaturase indices have been reported for HF in comparison to Belgian Blue cows. Jersey and Brown-Swiss cows had even lower values than the HF (Soyeurt et al. 2008).

Dissimilarity in the FA profile of HF as compared to Sahiwal despite being fed on the same diet is worth pondering. A significant proportion of variability in FA composition has been related to the animal genetics. Soyeurt et al. (2007) reported that genetics was responsible for one fifth of the variations described for milk fatty acid concentrations, particularly for the most plentiful FA. The genetic effect could be exerted by affecting enzymes of fatty acid pathway or via supply systems of fatty acids obtained from diet such as low and very low density lipoproteins (Myburgh et al. 2012).

Extensive system of management

All the FA indices presented lowest value for the indigenous cattle under extensive system of management (Table 2). The n-6/n-3 ratio (2.7) and AI (33.9) were significantly lower than that of cattle under intensive management. Similarly, they had different Δ^9-desaturase profile than that of farm managed Sahiwal. Significantly lower and higher values were obtained for C16:1 Δ^9-desaturase and C18:1 Δ^9-desaturase, respectively, as compared to Sahiwal. Whereas, C14:1 Δ^9-desaturase did not differ (Table 2). The n-6/n-3 ratio in bovine milk actually reflected the amount of linoleic acid (LA) in comparison to α-linolenic acid (ALA), as these correspond to the highest concentrated n-6

and n-3 FA. A lesser n-6/n-3 ratio was a consequence of fodder-based diet as cereals are rich in LA, whereas forage contains higher amounts of ALA (Schwendel et al. 2015). Accordingly, the dissimilarity in the FA indices of indigenous cows maintained under farm and those in the zero input system (Table 2) may very well be attributed to the feeding practices.

Mineral composition

Comparison of individual minerals was important as they might be advantageous for animals and humans or on the flip side might be considered as contaminants.

Intensive system of management

The results indicated absence of any statistically significant variation ($P < 0.05$) in the mineral composition of milk among Indian, exotic and crossbred cattle (Table 3). This finding was in accordance with the results described by Hurley (1997) which concluded mineral composition to be the among the slightest changeable bovine milk components across different breeds. The only exception was Na. Its mean ± SE values were significantly lower in Sahiwal (41.35 ± 0.90) as compared to HF (47.20 ± 1.55) and crossbred (48.35 ± 2.32) cattle. Similar to our results, only minor differences have been reported from South Africa, USA, UK, Denmark, Australia and New Zealand in the cow milk (Schonfeldt et al. 2012).

Extensive system of management

Significantly higher ($P < 0.05$) concentrations of Zn, Fe, P and Cu were present in the milk of grazing indigenous cattle (Table 3). Concentration of Na was even lower than that of Sahiwal. Milk of grazing indigenous cows may be considered as a rich source of minerals which play important role in metabolism and hence can positively contribute to consumer health. Phosphorous is required in large quantities by young ones for increase of bones and soft tissue masses, pH maintenance and energy conservation and utilization. The micro minerals (Cu, Fe and Zn) act as the co-factors for enzymes (Hurley 1997). Contributing factors for hypertension include obesity, sedentary life style, and high intake of Na in some individuals. Reduced level of Na in the milk of grazing cattle is thus an advantageous attribute for such persons. A recent meta-analyses, reported higher Fe concentration in organic milk (Srednicka-Tober et al. 2016), similar to our finding of higher Fe concentration in the milk of grazing cattle (Table 3). However, it is not expected to have a prominent role as milk is not considered to be a significant source of iron for human beings (Lim et al. 2013).

Concentration of Ca, Mg and K in the grazing cattle milk was similar to that recorded under intensive system of management (Table 3). Published literature also suggests that Ca and Mg content of milk are not affected to a large extent by the variation in the diet (van Hulzen et al. 2009).

Vitamin composition

Vitamin composition is summarized in Table 4.

Intensive system of management

Average concentrations of all the vitamins did not differ between farm managed *indicine* and *taurine* cattle, except for the slightly higher Vitamin E in Sahiwal cows

Table 3 Milk mineral profiles of Sahiwal, Holstein–Friesian and crossbred cows under intensive management and of indigenous cattle maintained under extensive system

Mineral (per 100 g of milk)	Sahiwal		Holstein–Friesian		Crossbred		Indigenous cattle		Level of significance
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	
Zinc (μg)	359.532^{a}	16.246	338.780^{a}	13.866	340.520^{a}	17.361	522.308^{b}	36.739	***
Iron (μg)	29.942^{a}	1.128	30.245^{a}	1.184	33.610^{a}	1.389	44.878^{b}	2.126	***
Sodium (mg)	41.353^{a}	0.896	47.195^{b}	1.554	48.348^{b}	2.323	25.490^{c}	1.749	***
Calcium (mg)	123.177	2.000	122.250	1.955	118.533	2.609	119.098	2.521	NS
Magnesium (mg)	13.538	0.410	12.638	0.672	13.420	0.371	13.328	0.598	NS
Potassium (mg)	143.387	3.921	140.080	5.979	142.895	4.363	146.078	5.463	NS
Phosphorus (mg)	97.168^{a}	1.876	98.020^{a}	3.296	96.345^{a}	3.573	108.988^{b}	0.997	*
Copper (μg)	9.732^{a}	0.494	9.975^{a}	0.528	10.988^{ab}	0.751	12.235^{b}	0.645	*

Each value represents the mean of observations on ten animals

NS non significant

Mean with different superscript(s) in a row differs significantly

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

(0.018 mg/100 g). Retinol content in the milk of farm managed cows in the current study (Table 4) was similar to that reported for USA (28 µg/100 g) and Denmark (29.2 µg/100 g), whereas, corresponding values were much higher for UK (52 µg/100 g) and South Africa (43.4 µg/100 g) (Schonfeldt et al. 2012).

Extensive system of management

All the vitamins, except vitamin B5 were higher in the milk of indigenous cow under extensive system of management (Table 4). Vitamin A, C, E and β-carotene were more than two folds higher. Elevated concentration of these four vitamins will be of importance to milk processing units. Being antioxidants, these will check emergence of oxidized flavor in the milk, which is expected due to the higher level of PUFA in the milk of grazing cattle (Table 2). In addition, increased intake of antioxidants is nutritionally desirable to reduce oxidative stress and hence can be beneficial in various chronic health conditions (Willcox et al. 2004). Moreover, yellow tint of cheese and butter caused by carotenoids of milk fat is an attractive attribute for the consumers. It is considered to be an indicator that the product has originated from cows thriving on pasture diets.

Studies till now have demonstrated that feed composition manipulate content of milk vitamins as well as their precursors. The concentration of β-carotene and α-tocopherol in the diet modified their contents in the milk (Mogensen et al. 2012). Fresh forage is the richest source of theses vitamins among animal feed (Schwendel et al. 2015). Preserved or desiccated forages and cereals are not considered to be as good as their fresh counterparts (Kay et al. 2005). Higher proportion of vitamins in the milk of indigenous cattle maintained under zero input system may be attributed to the grazing of these animals.

Amino acid profile

Milk protein consists of all the essential amino acids necessary for human beings and is an important source of high biological value protein. It is specifically important in developing countries like India, where rice or tubers are staples and diets are cereal based. Glutamic acid concentration was highest and was followed by Proline in all the groups in this study (Table 5). The next three amino acids as per declining concentrations were Leucine, Aspartic acid and Lysine in HF and crossbred milk, whereas for *Bos indicus* cattle (Sahiwal and extensive group) the order was Aspartic acid, Leucine and Lysine (Table 5).

Intensive system of management

Sahiwal milk had slightly higher total amino acids (g/100 g of milk) as compared to HF, whereas no difference was recorded with the crossbred cattle (Table 5). Total essential amino acids did not differ between the three groups managed at the farm. Significant difference ($P < 0.05$) was not observed in the amino acid profile of the Sahiwal and HF breed except for Histidine, Serine, Glycine, Lysine and Tyrosine. Histidine was lower in Sahiwal milk, whereas remaining four amino acids were present in higher

Table 4 Vitamin composition of milk from Sahiwal, Holstein–Friesian and crossbred cows under intensive management and of indigenous cattle in extensive system

Vitamin (per 100 g of milk)	Sahiwal		Holstein–Friesian		Crossbred		Indigenous cattle		Level of significance
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	
Vitamin A (as Retinol, acetate palmitate) (µg)	29.203[a]	1.030	31.398[a]	0.732	30.923[a]	2.006	60.015[b]	2.692	***
Vitamin E (mg)	0.018[a]	0.002	0.013[b]	0.001	0.010[b]	0.000	0.078[c]	0.002	***
β Carotene (µg)	192.218[a]	8.752	179.309[a]	10.444	178.125[a]	9.781	440.431[b]	15.251	***
Vitamin B1 (mg)	0.050[a]	0.003	0.042[a]	0.006	0.047[a]	0.003	0.065[b]	0.003	*
Vitamin B3 (mg)	0.072[a]	0.005	0.079[a]	0.003	0.080[a]	0.004	0.105[b]	0.008	**
Vitamin B5 (mg)	0.311	0.009	0.323	0.012	0.340	0.011	0.352	0.009	NS
Vitamin B6 (mg)	0.040[a]	0.002	0.042[a]	0.002	0.050[b]	0.002	0.053[b]	0.004	**
Vitamin B2 (µg)	0.171[a]	0.005	0.173[a]	0.005	0.165[a]	0.005	0.235[b]	0.004	***
Vitamin C (mg/100 ml of milk)	0.182[a]	0.016	0.164[a]	0.017	0.135[a]	0.016	0.527[b]	0.052	***

Each value represents the mean of observations on ten animals

NS non significant

Mean with different superscript(s) in a row differs significantly

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

Table 5 Amino acids (g/100 g of milk) in the milk of Sahiwal, Holstein–Friesian and crossbred cows maintained in intensive and indigenous cattle under extensive system of management

Amino acid	Sahiwal		Holstein–Friesian		Crossbred		Indigenous cattle		Level of significance
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	
Histidine	0.060^{a}	0.003	0.072^{b}	0.004	0.066^{ab}	0.002	0.065^{ab}	0.002	*
Serine	0.120^{a}	0.005	0.095^{b}	0.007	0.110^{ab}	0.005	0.165^{c}	0.004	***
Arginine	0.094	0.003	0.088	0.007	0.099	0.009	0.087	0.004	NS
Glycine	0.104^{a}	0.007	0.086^{b}	0.006	0.085^{b}	0.007	0.124^{c}	0.005	***
Aspartic acid	0.296^{ab}	0.022	0.256^{a}	0.019	0.262^{a}	0.016	0.329^{b}	0.012	*
Glutamic acid	0.713^{a}	0.024	0.659^{ab}	0.024	0.648^{b}	0.018	0.788^{c}	0.014	***
Threonine	0.138^{a}	0.002	0.143^{a}	0.003	0.141^{a}	0.004	0.159^{b}	0.006	**
Alanine	0.098	0.002	0.096	0.003	0.100	0.004	0.093	0.006	NS
Proline	0.342^{a}	0.011	0.340^{a}	0.005	0.339^{a}	0.006	0.396^{b}	0.023	*
Cysteine	0.004^{a}	0.000	0.003^{a}	0.000	0.003^{a}	0.000	0.006^{b}	0.001	***
Lysine	0.231^{a}	0.007	0.207^{b}	0.005	0.221^{ab}	0.004	0.252^{c}	0.005	***
Tyrosine	0.151^{a}	0.004	0.135^{b}	0.002	0.144^{ab}	0.002	0.170^{c}	0.005	***
Methionine	0.060^{a}	0.003	0.053^{a}	0.003	0.061^{a}	0.004	0.074^{b}	0.002	***
Valine	0.171^{a}	0.004	0.181^{ab}	0.004	0.187^{b}	0.006	0.213^{c}	0.004	***
Isoleucine	0.159^{a}	0.005	0.151^{a}	0.004	0.162^{a}	0.004	0.208^{b}	0.007	***
Leucine	0.266^{a}	0.010	0.264^{a}	0.005	0.272^{a}	0.006	0.316^{b}	0.013	**
Phenylalanine	0.148^{a}	0.003	0.139^{a}	0.003	0.140^{a}	0.004	0.169^{b}	0.004	***
Essential Amino acids	1.233^{a}	0.016	1.210^{a}	0.014	1.250^{a}	0.019	1.456^{b}	0.017	***
Total amino acids	3.167^{a}	0.079	2.968^{b}	0.050	3.039^{ab}	0.033	3.614^{c}	0.057	***

Each value represents the mean of observations on ten animals. Essential amino acids are in bold

NS non significant

Mean with different superscript(s) in a row differs significantly

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

concentrations (Table 5). Similarly, Mapekula et al. (2011) reported small differences in the amino acid composition of milk derived from two breeds of cattle and their crossbreds. Variation in milk amino acid concentrations of the indigenous cattle of Bangladesh (*Bos indicus*) and Holstein crossbred has also been reported (Islam et al. 2014). Bangladesh cattle had higher concentration of Threonine, Alanine and Aspartic acid as compared to Holstein crossbred.

Extensive system of management

Total amino acid content was high in the milk of grazing indigenous cattle (Table 5). Likewise, significantly higher ($P < 0.05$) essential amino acids (1.46 ± 0.02) could be observed. All the amino acids except Histidine, Arginine and Aspartic acid were elevated (Table 5). One can expect variation in the milk amino acid composition in different management systems (extensive and intensive) as majority of the amino acids in the mammary gland are obtained from the cow's diet. Krizova et al. (2013) also reported difference in the amino acid spectrum of milk obtained from Czech Fleckvieh and Holstein breed under grazing and non-grazing feeding system. Published literature also supports difference in amino acid content due to feeding changes (Schonfeldt et al. 2012). As cows of all the three groups were fed on similar diet at the farm, the observed variation of Sahiwal and HF amino acids could very well be because of the species difference (*taurus* vs *indicus*). Difference in the composition of feed between farm and forest grazing could be the underlying reason for the increased milk amino acids under extrinsic system.

Conclusion

Milk of Sahiwal cattle had slight edge over Holstein because of higher total solids, lactose, fat and favorable fatty acid composition. Local cattle maintained exclusively on extensive system produced milk with more favorable characteristics from a human health perspective. Their milk was a better source of protein, amino acids, fat, nutritionally desirable fatty acids, vitamins and minerals. Higher milk productivity was never the reason why Indian zebu

cows (*Bos indicus*) were revered in India as it is the quality of milk that has been the talk of folklore. It is possible that almost total pasture feeding in ancient times was responsible for proclaimed health promoting qualities of the milk. Promising future for indigenous cows maintained on grazing can be expected in the current scenario where organic milk production with particular emphasis on grazing is gaining momentum throughout the world. Additional studies are required to substantiate the results. Nevertheless, the data generated supplements the very meager evidence base available for comparison of cow milk composition in India. It will set a benchmark for future investigations on the milk composition of indigenous cattle.

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