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Fat Soluble Vitamins Profile of Milk in Various Indigenous Cattle Breeds of Rajasthan

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ABSTRACT

The present study was undertaken to investigate the fat soluble vitamins profile in milk of zebu cattle viz. Kankrej, Sahiwal and Rathi in hot arid region of India. About 150 indigenous milking cattle (50 from each breed) viz. Kankrej, Sahiwal and Rathi were included in the study and 100 ml of milk sample was collected from four quarter of udder of lactating cows as pool sample from different Livestock research stations of Rajasthan University of Veterinary and Animal science, Bikaner. Milk samples were analyzed by HPLC and one way ANOVA and DMRT (SPPS 24) to assess fat soluble vitamins profile in milk. The results showed significant variation of retinol content ($p<0.05$) among three cattle breeds. Whereas, Sahiwal cattle have significantly higher retinol content as compared to Kankrej and Rathi. α-tocopherol, cholecalciferol and vitamin D_2 were significantly not differ in among breeds. The present study concluded the milk from the indigenous cattle breeds have distinct compositions and quantities of different active components that can be harnessed to promote different functional foods based on indigenous cattle milk.

HIGHLIGHTS

- Variation of fat soluble vitamins profile in milk of different Zebu cattle breeds.
- Sahiwal cattle have significantly higher retinol content as compared to Kankrej and Rathi.

Keywords: Fat soluble vitamins, Retinol, α-tocopherol, Cholecalciferol, Indigenous cattle, Milk

Milk is a wealthy source of nutrients and is considered a valuable element of a complete diet (Khan *et al.,* 2019). Milk, a lacteal secretion from a healthy lactating animal, contains various nutrients, including proteins, carbohydrates, fats, minerals, and vitamins, and thus possesses a wide range of nutritional and purposeful properties. Although, the milk composition of any species is based on the requirement of the neonate (Park, 2009; McGrath *et al.,* 2016). Moreover, it has also been explored

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for supplementary health benefits for infants, children, and adults of mammalian species, beyond their basic nutritional role (Park, 2009). Milk and milk products are nutritious food items containing several indispensable bioactive components such as oleic acid, conjugated linoleic acid, omega-3 fatty acids, vitamins, minerals and bioactive compounds such as antioxidants (Saxelin *et al.,* 2003). The bioactive components are the compounds either naturally existing in food or conceived during processing that may have physiological and biochemical functions when consumed by humans and have crucial impacts on human metabolism and health (Camara *et al.,* 2021). Earlier, various authors have suggested that the richest bioactive component in milk is the milk protein fraction, especially κ – casein, α – lactalbumin, β - lactoglobulin and whey proteins. Followed by fat and fatty acid fraction, viz., monoacylglycerols, saturated and unsaturated fatty acids, free fatty acids, cholesterol and A, D, E, K vitamins as bioactive components (Park, 2009). Previously, various workers demonstrated that these vitamins possess imperative biological functions, i.e. antimicrobial, anti-carcinogenic, anti-atherosclerosis, anti-hypertensive, antioxidative, anti-cytotoxic immune-modulator activities (Cicero *et al*., 2017).

The previous literature review has shown that exotic cattle, sheep and goat milk have fat soluble vitamin components; however, information regarding the fat soluble vitamin components of Indigenous cattle breeds' milk has not been previously explored. Therefore, the present study intended to explore the fat soluble vitamin components in the milk of various indigenous cattle breeds, viz. Kankrej, Sahiwal and Rathi were reared in the hot-arid region of Rajasthan, India.

MATERIALS AND METHODS

The study was performed at different university cattle farms, RAJUVAS, Bikaner, and Rajasthan, India. The Bikaner district of Rajasthan, India, is situated at 230 meters from sea level. Latitude and longitude position is 28° 01' 00''N and 73° 18' 43''E. The city comes in the middle of the Thar Desert and has a hot semi-arid region. During summer, high temperatures can exceed 48°C and during the winter comes below freezing.

Experimental animals

For this study, 36 indigenous lactating cattle breeds, *viz.,* Kankrej, Sahiwal and Rathi, were randomly selected (12 from each breed) and reared under similar feeding and housing management conditions. The animals were housed under a loose housing system having covered and open paddocks. The nutrient requirement of the animals was fulfilled by providing dry fodder, concentrate and *ad-libitum* green fodder. Full hand milking was practiced in both morning (4.00 to 5.00 am) and evening (3.30 to 4.30 pm) milking.

Collection of milk samples

Representative milk samples were collected from all the four quarters of lactating cows as pool samples. About 100 ml of pooled milk samples of all individual cows have been collected aseptically in the sterilized sampling bottle after discarding the first 4-5 streaks of foremilk.

Estimation of vitamins in milk

The solvent used for the experiment were methanol, acetonitrile and hexane with gradient grade HPLC by Sigma-Aldrich. The reagent was absolute ethanol of analytical grade. The vitamin standards were Ergocalciferol (vitamin D_2), Cholecalciferol (vitamin D_3), α-Tocopherol (vitamin E) and Retinol (vitamin A) by Sigma Aldrich. A Thermo-Scientific UHPLC system equipped with UltiMate™ HPG-3400SD standard binary pump, UltiMate™ ACC-3000 autosampler (1 to 200 µL), UltiMate™ DAD 3000 detectorwas used. Chromatographic separation was done on a Thermo Scientific™ Acclaim™ 120 C18 column (150 mm × 4.6 mm × 5 µm). Data were integrated using Thermo Scientific™ Chromeleon™ 7.2 Chromatography Data System (CDS) software. The mobile phase used was methanol (100%). The injection volume was set at 10 µL. The wavelength used to detectvitamin D_2 and D_3 was 265 nm, for vitamin E 294 nm, and for vitamin A it was 325 nm. The column temperature was held at 30ºC for vitamin D_2, 35ºC for vitamin D_3, 40ºC for vitamin E and 35ºC for vitamin A. The flow rate of the mobile phase was the same for all the vitamins analysis, i.e. 1 ml perminute.

Preparation of standard solutions for method validation

Chromatographic peaks were obtained for samples and identified by comparing them with standards. The working standard stock solution (c = 1 mg/ml) was prepared by dissolving 5.0 mg of vitamin D_2, D_3, E and A into 5.0 ml methanol in a volumetric flaskon each day of the validation. Three calibrationstandards viz. 0.500 mg/ml, 0.250 mg/ml and 0.125 mg/ml were prepared for each standard calibration curve. Appropriate volumes of the working standard solution were prepared intoseparate amber vials. The calibration curve was constructed by plotting the peak area of the standard against its analysis.

Extraction

The extraction of vitamin D_2, D_3, E and A was done according to Rodas- Mendoza *et al.* (2003) with some modifications. First, the sample was prepared by adding 4 ml of absolute ethanol into 1 ml of milk sample in a centrifuge tube. The mixture was then vortex for 2 minutes. After that, 400 µL hexane was added to the mixture, and again it was vortex for 4 minutes for proper mixing of the solution. After that, it was centrifuged at 2500 rpm for 5 minutes at room temperature. Consecutively, the supernatant was taken and evaporated at 30°C in a vacuum concentrator. After evaporation of the solution, 1 ml methanol was added to the residue. It was then filtered with 0.45 µm filtered and put in the HPLC for analysis.

STATISTICAL ANALYSIS

The effect of breeds on vitamins was analyzed by analysis of variance. The data were examined by the General Linear Model Procedure of SPSS software statistical package (24.0), and means were contrasted by Duncan's multiple range test.

RESULTS AND DISCUSSION

Fat-Soluble Vitamins Profile

We different fat-soluble vitamins, viz. Retinol (Vitamin A), α-Tocopherol (vitamin E), Ergocalciferol (vitamin D_2) and Cholecalciferol (vitamin D_3) were analyzed in milk from different cattle breeds to determine significant differences. The results of the standard graph of vitamin A, D_2, D_3 and E are shown in fig. 1, 2, 3 and 4, respectively. The retention times of vitamin A, D2, D3 and E were 2.96, 7.51, 6.24 and 8.43.

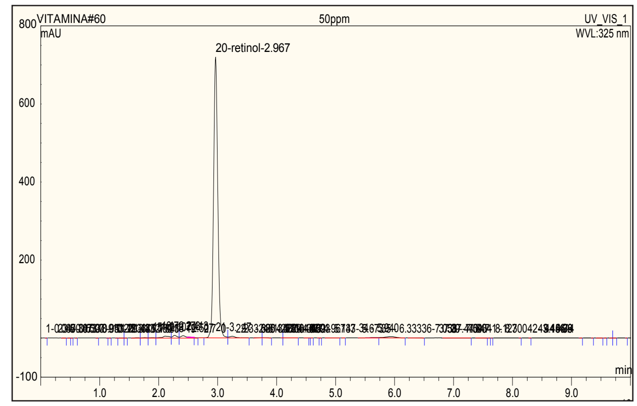

Fig. 1: Standard graph of vitamin A

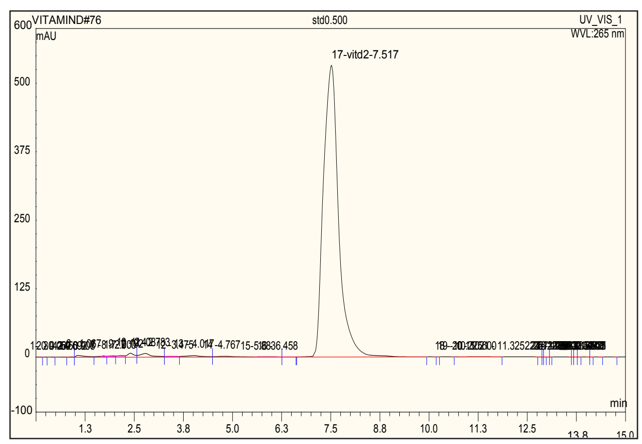

Fig. 2: Standard graph of vitamin D_2

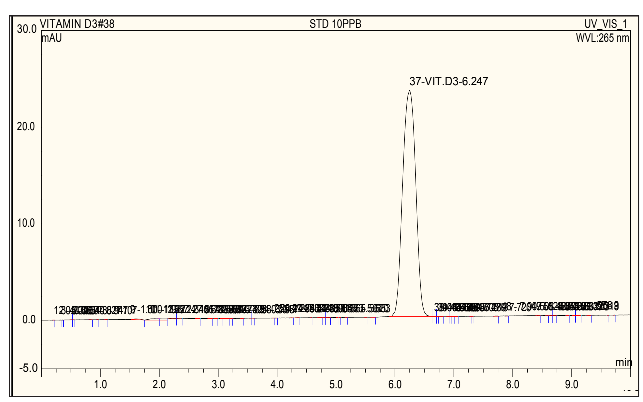

Fig. 3: Standard graph of vitamin D_3

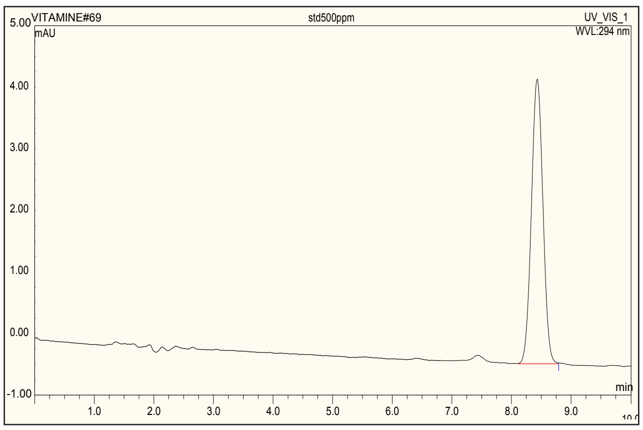

Fig. 4: Standard graph of vitamin E

Retinol

In cow milk, vitamin A is present as free Retinol, retinol esters, and carotene. The concentration of free Retinol was measured in the milk of different indigenous cattle breeds, and the Mean ± SE values of Retinol are presented in the table 1. In the analysis of variance (ANOVA) and Post-hoc Duncan's Multiple Range Test, the pair-wise comparison revealed that retinol content between different breeds differed (p<0.05). Sahiwal has shown higher (P =) retinol content than Kankrej and Rathi. The concentration of vitamin A is affected by variable factors such as feeding (diet), environment (season), stage of lactation, heat treatment and genetic factors (breeds and species). Raw milk samples were randomly collected from different breeds kept in similar feeding and environment conditions. These differences might be due to genetic effects (breeds). This is following other studies that showed significant differences in vitamin A concentration in milk from different cattle breeds, viz. Polish Holstein-Friesian (0.359 ± ± 0.071 mg/l), Montbéliarde (0.414 ± 0.080 mg/l), Jersey (0.398 ± 0.094 mg/l) and Simmental (0.465 ± 0.077mg/l) (Krol *et al.,* 2017). Ramalho *et al.* (2012) found the level of free Retinol higher in Minhota 0.22 ± 0.15 µg/g of fat as compared to Holstein (0.13 ± 0.08 µg/ g of fat. Joytika (2016) observed that the Karan fries have a higher level of Retinol than Sahiwal and Tharparkar.

Concerns regarding α-tocopherol content and mean values are presented in the table 1. The analysis of variance (ANOVA) revealed that α-tocopherol content between different indigenous cattle breeds was non-significant. Based on Post-hoc Duncan's Multiple Range Test, the pair-wise comparison revealed that the Sahiwal cattle breed has higher (p<0.05) α-tocopherol content than Rathi. This is following other studies in the literature that showed a significant difference of α-tocopherol concentration in milk < UNK>- tocopherol concentration from different cattle breeds, viz. Polish Holstein-Friesian (1.106 ± 0.299 mg/l), Montbéliarde (1.243 ± 0.338 mg/l) Jersey (1.199 ± 0.284 mg/l) and Simmental (1.302 ± 0.310 mg/l). Among different cattle breeds, the concentration of α-tocopherol was found higher in milk of Minhota cattle breed than Holstein cattle breed (Król *et al.,* 2017). In addition, various workers reported genetic variation of α-tocopherol content in milk of different species (Sunaric *et al.* 2012; Revilla *et al.* 2014). Sahiwal cattle have higher Vitamin E contents than Kankrej and Rathi. This fact could be related to Sahiwal cattle's metabolism and its greater secretory capacity for Vitamin E. Therefore, as vitamin E is considered an important natural antioxidant, they increase protection against oxidative lipid damage and, consequently, increase the shelf-life of milk.

Table 1: Means ± SE different fat soluble Vitamins in milk from different cattle breeds

Assay	Kankrej	Rathi	Sahiwal
Vitamin A (µg/ml)	0.43 ± 0.04^{a}	0.44 ± 0.04^{a}	0.59 ± 0.07^{b}
Vitamin E(µg/lt)	0.55 ± 0.05^{ab}	0.47 ± 0.06^{a}	0.67 ± 0.08^{b}
Vitamin D_2 (µg/lt)	0.68 ± 0.07^{a}	0.62 ± 0.05^{ab}	0.48 ± 0.05^{b}
Vitamin D_3(µg/lt)	0.63 ± 0.14^{ab}	0.93 ± 0.15^{a}	0.49 ± 0.09^{b}

a, b, c - differences significant at P<0.05.

The concentration of Cholecalciferol and Ergocalciferol was presented in table 1. The Mean ± SE values of Cholecalciferol and Ergocalciferol content in milk were 0.63 ± 0.14 and 0.68 ± 0.07ng/ml, 0.93 ± 0.15 and 0.62 ± 0.05 ng/ml, 0.49 ± 0.09 µg/ml and 0.48 ± 0.05ng/ml in Kankrej, Rathi and Sahiwal, respectively. Analysis of Variance (ANOVA) revealed no significant difference in Cholecalciferol and Ergocalciferol levels among different indigenous cattle breeds. But based on Post-hoc Duncan's Multiple Range Test, the pair-wise comparison revealed that Rathi cattle breeds had shown significantly (P<0.05) higher Cholecalciferol content than Sahiwal but no significant difference between Kankrej and the other two breeds. On the other hand, Kankrej cattle breeds significantly ($p<0.05$) higher Ergocalciferol content than Sahiwal, but there was no significant difference between Rathi and the other two breeds. Thus, other studies in the literature showed significant differences in vitamin D_3 concentration in milk from different cattle breeds, viz. Polish Holstein-Friesian (0.589 ± 0.106μg/l), Montbéliarde (0.696 ± 0.157 μg/l) Jersey (0.620 ± 0.170 μg/l) and Simmental (0.653 ± 0.113 μg/l) (Król *et al.,* 2017). Whereas according to Ramalho *et al.* (2012), levels of vitamin D_3 were non significantly differed between Milhota and Holstein cattle breeds. The present study results are similar to those reported in different literature for different cattle breeds (Król *et al.,* 2017).

It is worth noted that less milk should be part of the human diet, but fat-soluble vitamins are more valuable for human health. So, it is important to find out which breeds produced more concentration of particular nutrients required by human or individual person and take the milk of these breeds. Looking at the overall content of all milk components evaluated, milk from Sahiwal cattle had higher levels of Retinol and α-tocopherol which are helpful for human health.

CONCLUSION

In present study, it was observed that breeds factor significantly affect milk quality. Milk from the indigenous cattle breeds have a distinct composition and possess varied types and amounts of vitamine components and enzymes, which can be harnessed to promote different functional foods based on indigenous cattle milk. Further pharmaceutical preparations can be promoted for the benefit of farmers and entrepreneurs. Breed difference has a pronounced effect on milk fat-soluble vitamins, which is an important observation regarding future genetic selection plans.

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