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The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(5): 218-223

www.thepharmajournal.com
Received: 27-02-2023
Accepted: 30-03-2023

D Deepak
College of Veterinary and Animal Science, Sardar Vallabhbhai Patel University of Agriculture & Technology, Modipuram, Meerut, Uttar Pradesh, India

UK De
Division of Veterinary Medicine, ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh, India

Ahmad Fahim
College of Veterinary and Animal Science, Sardar Vallabhbhai Patel University of Agriculture & Technology, Modipuram, Meerut, Uttar Pradesh, India

Ram Kumar Singh
College of Veterinary and Animal Science, Sardar Vallabhbhai Patel University of Agriculture & Technology, Modipuram, Meerut, Uttar Pradesh, India

Corresponding Author:
Ram Kumar Singh
College of Veterinary and Animal Science, Sardar Vallabhbhai Patel University of Agriculture & Technology, Modipuram, Meerut, Uttar Pradesh, India

Effect of micronutrient antioxidant administration around peri-parturient period on milk composition of murrah buffaloes (*Bubalus bubalis*)

D Deepak, UK De, Ahmad Fahim and Ram Kumar Singh

Abstract
Buffalo milk is blessed with illustrious nutritive profile *viz*. richer in energy, protein, vitamin and mineral content and is also one of the most consumed dairy products. Higher nutritive profiles make it as preferable food for consumption and processing to other nutraceutical dairy products and also predisposes buffalo milk for quick development of microorganisms. This predisposition to infection is further reinforced by high susceptibility of periparturient period for high bacterial infection of milk and mammary gland which ultimately affects milk quality. Existing animal husbandry feeding practices are predisposing livestock to the deficiency of micronutrient antioxidants. Enhanced oxidative stress in association to deficient micronutrient antioxidants and negative energy balance during transition period can compromise milk composition. Therefore, the present study was performed to study the effect of administration of micronutrient antioxidants around periparturient period on milk composition of murrah buffaloes. Clinically healthy twenty four buffaloes in their last month of gestation were selected and redistributed into three groups *viz*. control, VE and MM. VE and MM groups were administered with injection of Vitamin E & selenium & multiminerals respectively on 30th day of prepartum, 7th day of prepartum and day 2 of postpartum. The milk sample was collected on day 1, day 7, day 14 and day 28 of calving. The investigations revealed a significant increase (p<0.001) in milk fat content on day 1 of calving in the Vitamin E and selenium administered group as compared to the control group. No significant change in milk SNF, lactose, protein and pH of milk were observed between control and supplemented groups. Finally, the study concluded that the parenteral administration of vitamin E and selenium during the periparturient period improves the composition of buffalo milk.

Keywords: Buffalo, milk, peri-parturient, micronutrient and antioxidants

Introduction
India has been the leading milk producer of the world since the last two decades (DAHDF, 2020) [7]. Approximately 200MT of milk is primarily harvested through buffaloes (BAHS, 2022; Singh *et al*., 2021) [3, 28]. Since the animal husbandry sector of India is not an organized sector and majority of stakeholders are marginal income farmers (Hegde, 2019). Marginal farmers follow the traditional lignocellulose rich feeding practices and are reluctant to adopt the scientific feeding regimen due to deficient knowledge regarding scientific feeding (Sharma, 2015) [26]. Moreover, the traditional feeding practices don't emphasis upon ration fortification with micronutrient antioxidants even during the harsh scenario of periparturient period and ultimately animals during the periparturient period may severely suffer deficient levels of these micronutrient antioxidants in the body. Deficiency of micronutrients can precipitate adverse effects on the health of animals and compromise the milk production (Mudgal *et al*., 2014) [20].
Administration of Vitamin E and selenium in late gestation period is known to have beneficial effects on milk production, postpartum fertility and incidence of mastitis (Qureshi *et al*., 2010; Gangwar *et al*., 2008) [22, 8]. Similarly mineral administration is also known to boost milk production in buffalo (Madke *et al*., 2018; Rupasi *et al*., 2013) [18, 24]. These micronutrient antioxidants can aid in the smooth transition of buffalo from dry stage of late gestation to lactation state (Hoque *et al*., 2016) [13]. Therefore, keeping in mind the aforementioned limitations of traditional feeding practices and adverse effect of on milk quality attributes and benefits of micronutrient antioxidant administration, present study was performed to investigate the effect of multiple strategic administration of micronutrient antioxidants around periparturient period on the composition of colostrum and milk of murrah buffaloes.

Materials and Methods

Animal's selection & feeding regimen

Twenty four murrah buffaloes of similar parity and body weight were selected from Livestock Research Centre, Sardar Vallabhbhai Patel University of Agriculture & Technology, Modipuram, Meerut- 250110 (28.98450N, 77.70640E) having IAEC approval No.V-11011 (13)/3/2022-CPCSEA-DADF). Selected buffaloes were falling in their last trimester of gestation and were redistributed into three groups (n=8) *viz* control (GP-I), VE (GP- II) and MM (GP-III). The control group was provided with gestation ration and lactation ration as per the recommendation of NRC, 2001 for buffaloes weighing 364.700 kg (± 16.625). In addition to gestation and lactation ration, VE and MM group were administered with vitamin E- selenium injection containing DL α- Tocopherol acetate 50mg & Sodium selenite 1.5 mg/mL at the dose rate of 2mL/45 Kg BW by subcutaneous route and multimineral injection containing as disodium copper EDTA 15.0 mg, disodium manganese EDTA 10 mg, sodium selenite 5.0 mg, and 40.0 mg disodium zinc EDTA per ml at the dose rate of 1.0 ml/ 100 kg b.wt subcutaneous injection respectively on day 30th prepartum, day 7th prepartum and day 2th postpartum. Moreover, 5.0kg dry roughage and ad- lib green fodder were provided for experimental animals. The chemical composition of concentrate mixture offered during experiment is having crude protein (21%), crude fiber (12%), crude fat (2.6%), sand silica (4.0%), urea (1.0%), NaCl (2.0%), phosphorus (0.5%,) calcium (0.5%), total digestible nutrients (68.70%) and Vitamin A (5000IU/Kg).

Procurement of supplements

All the injectable supplements used in the present study *viz*. vitamin E and selenium injection (*Repronol*, *Cadila*, *Ahmedabad*, *India*) and multiminerals (*Stimvet*, *Safecon lifesciences*, *Uttarakhand*, *India*) were procured from a reputed supplier.

Analysis of milk quality attributes

The milk samples were collected on the 1st day of calving, day 7th, day 14th and day 28th postpartum. The composition of milk was analysed using automated milk analyser (*Lactoscan*, *Bulgaria*) available in the Department of Livestock Production and Management of College of Veterinary and Animal Sciences, SVPUAT, Meerut-250110, India.

Statistical analysis

Analysis of data was done by using SPSS (version 20sss) one-way ANOVA for milk parameters. Data are presented as means ± SD and considered significant at $p<0.05$.

Results

Investigations of milk fat (%) content (Table 1 and figure 1) revealed the range of 6.188 ±0.055% to 6.650±0.042%, 6.225±0.037% to 8.400±0.529% and 6.163±0.026% to 6.588±0.029% for control, VE and MM group respectively from day 1 of calving to day 28 postpartum. In all the groups, significantly lower value of milk fat was observed on day 1 of calving as compared to day 7, day 14 and day 28 postpartum. Comparing between groups, the value of milk fat was significantly ($p<0.001$) higher in the VE supplemented group as compared to the control and MM group on day 1 of calving. The mean value milk solid non-fat (SNF) content (Table 2 ad figure 2) ranged from 11.344±0.255% to 13.518±0.533%, 11.275 ±0.327% to 14.323±0.349% and 10.955±0.262% to 13.365±0.551% in control, VE and MM administered groups respectively, from day 1 of calving to day 28 postpartum. In control group, significantly ($p<0.001$) higher value of SNF was observed on day 1 of calving as compared to day 7, day 14 and day 28 postpartum while the value of SNF on day 1 of calving was significantly ($p<0.001$) higher than day 14 and day 28 postpartum for VES and MM group. No significant variation (P=0.05) in the value of SNF was observed comparing between control and supplemented groups. The percent value of lactose (Table 3 and figure 3) in milk were 3.724±0.007% to 5.479±0.018%, 3.738±0.011% to 5.484±0.026% and 3.746±0.010% to 5.479±0.037% for control, VE and MM group respectively from day 1 of calving to day 28 postpartum. In control and supplemented groups, mean value of lactose on day1 of calving was significantly ($p<0.001$) lower than day 7, day 14 and day 28 postpartum. When the value of milk lactose of the control group was compared with the value of lactose in the supplemented group, no significant variation in the value of lactose (%) was observed from day 1 of calving to day 28 postpartum. The values of milk protein percentage (Table 4 and figure 4) were in the range of 4.299±0.015% to 8.611±0.019%, 4.26±0.014% to 8.606±0.023% and 4.259±0.016% to 8.603±0.023% in control, VE and MM administered groups, respectively from day 1 of calving to day 28 postpartum. The mean value of protein on day 1 of calving was significantly ($p<0.001$) higher than day 7, day 14 and day 28 postpartum for control and supplemented groups. When the value of milk protein of the control group was compared with the value of milk protein in the supplemented group, no significant (P=0.05) variation was observed between control and supplemented groups. Finally the pH of milk (Table 5 and figure 5) during the experimental period were in the range of 6.438±0.008 to 6.5878±0.048, 6.421±0.004 to 6.546±0.011 and 6.441±0.009 to 6.54±0.009 in control, VE and MM administered groups, respectively from day of calving to day 28 postpartum. The pH of milk on day 1 of calving was significantly ($p<0.001$) higher than day 7, day 14 and day 28 postpartum in control and supplemented groups. When the mean value of milk pH for the control group was compared with the milk pH of the supplemented group, no significant (P=0.05) variation in the pH of milk was observed between control and supplemented groups from day 1 of calving to day 28 postpartum.

Table 1: Changes in milk fat (%) in Vitamin E and selenium (VE) and multi-mineral (MM) supplemented groups as compared to control

Group	Day 1	Day 7	Day 14	Day 28	Group Mean	P value
Control	6.350^{Ax}±0.059	6.650^{ABy}±0.042	6.213^{Axy}±0.052	6.188^{Ay}±0.055	6.350±0.234	<0.001
VE	8.400^{Bx} ±0.529	6.863^{By}±0.0156	6.225^{Ay}±0.037	6.250^{Ay}±0.05	6.934±0.206	<0.001
MM	6.588^{Ax} ±0.029	6.500^{Ay}±0.068	6.163^{Ax}±0.026	6.238^{Ax}±0.053	6.372±0.039	<0.001
Day mean	7.003±0.255	6.671±0.064	6.200±0.022	6.225±0.029		
P value	<0.001	0.059	0.508	0.680		

Data are presented as means ± SD and considered significant at $p<0.05$

Table 2: Changes in milk SNF (%) in Vitamin E and selenium (VE) and multimineral (MM) supplemented groups as compared to control

Group	Day 0	Day 7	Day 14	Day 28	Group Mean	P value
Control	13.518^{Ax}±0.533	11.791^{Ay}±0.449	11.661^{Ay}±0.052	11.344^{Ay}±0.255	12.079±0.208	<0.001
VE	14.323^{Ax}±0.349	11.988^{ABy}±0.170	11.850^{Ay}±0.131	11.275^{Ay}±0.327	12.359±0.244	<0.001
MM	13.365^{Ax}±0.551	11.611^{Ay}±0.055	11.608^{Ay}±0.088	10.955^{Ay}±0.262	11.885±0.218	<0.001
Day mean	13.735±0.282	11.797±0.067	11.706±0.057	11.191±0.160		
P value	0.346	0.064	0.197	0.592		

Data are presented as means ± SD and considered significant at $p<0.05$.

Table 3: Changes in milk Lactose (%) in Vitamin E and selenium (VE) and multimineral (MM) supplemented groups as compared to control

Group	Day 0	Day 7	Day 14	Day 28	Group Mean	P value
Control	3.724^{Ax}±0.007	4.248^{Ay}±0.008	5.479^{Az}±0.018	5.423^{Az}±0.017	4.718±0.136	<0.001
VE	3.738^{Ax}±0.011	4.288^{Ay}±0.016	5.481^{Az}±0.015	5.484^{Az}±0.026	4.748±0.137	<0.001
MM	3.746^{Ax}±0.010	4.288^{By}±0.029	5.440^{Az}±0.012	5.479^{Az}±0.037	4.738±0.135	<0.001
Day mean	3.736±0.006	4.274±0.011	5.467±0.009	5.461±0.016		
P value	0.262	0.267	0.126	0.176		

Data are presented as means ± SD and considered significant at $p<0.05$.

Table 4: Changes in milk Protein (%) in Vitamin E and selenium (VE) and multimineral (MM) supplemented groups as compared to control

Group	Day 0	Day 7	Day 14	Day 28	Group Mean	P value
Control	8.611^{Az}±0.019	4.304^{Ay}±0.025	4.299^{Az}±0.015	4.299^{Az}±0.023	5.353±0.338	<0.001
VE	8.606^{Ax}±0.023	4.291^{Ay}±0.021	4.261^{Ay}±0.023	4.26^{AAy}±0.014	5.364±0.336	<0.001
MM	8.603^{Ax}±0.023	4.315^{Ay}±0.027	4.259^{Ay}±0.027	4.259^{Ay}±0.016	5.367±0.336	<0.001
Day mean	8.607±0.012	4.297±0.012	4.273±0.012	4.280±0.011		
P value	0.961	0.069	0.973	0.238		

Data are presented as means ± SD and considered significant at $p<0.05$.

Table 5: Changes in milk pH in Vitamin E and selenium (VE) and multimineral (MM) supplemented groups as compared to control

Group	Day 0	Day 7	Day 14	Day 28	Group Mean	P value
Control	6.438^{Ax}±0.008	6.538^{Ay}±0.008	6.5878^{Ay}±0.048	6.564^{Ay}±0.038	6.532±0.018	0.011
VE	6.421^{Ax}±0.004	6.521^{Ay}±0.003	6.546^{Ayz}±0.011	6.530^{Az}±0.005	6.504±0.009	<0.001
MM	6.441^{Ax}±0.009	6.541^{Ay}±0.009	6.540^{Ay}±0.011	6.531^{Ay}±0.008	6.513±0.009	<0.001
Day mean	6.433±0.004	6.533±0.005	6.558±0.017	6.542±0.013		
P value	0.150	0.150	0.478	0.498		

Data are presented as means ± SD and considered significant at $p<0.05$.

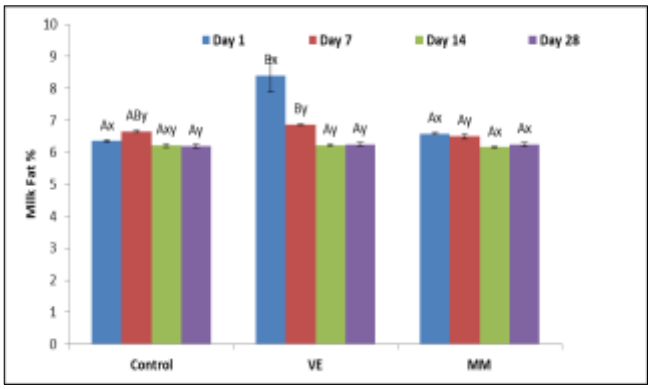

Fig 1: Changes in milk fat (%) in Vitamin E and selenium (VE) and multimineral (MM) supplemented groups as compared to control

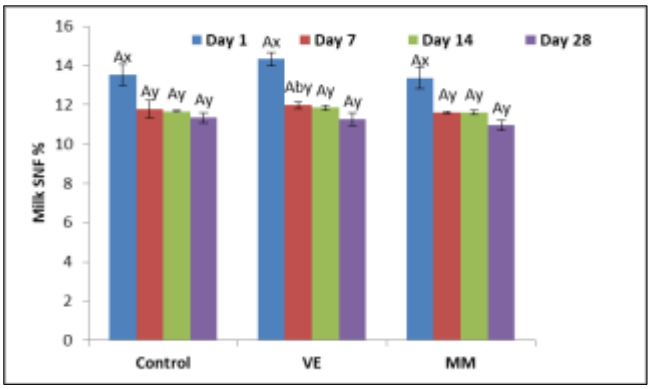

Fig 2: Changes in milk SNF (%) in Vitamin E and selenium (VE) and multimineral (MM) supplemented groups as compared to control

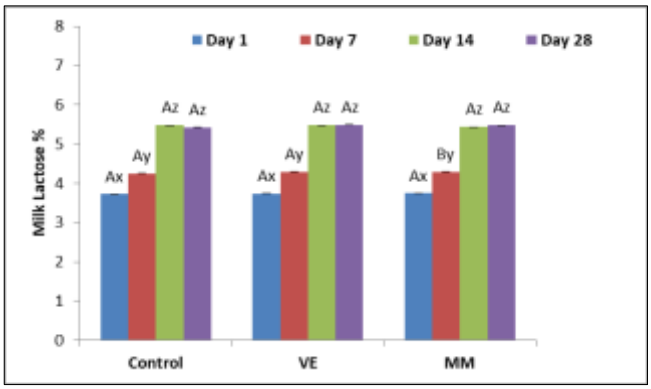

Fig 3: Changes in milk Lactose (%) in Vitamin E and selenium (VE) and multimineral (MM) supplemented groups as compared to control

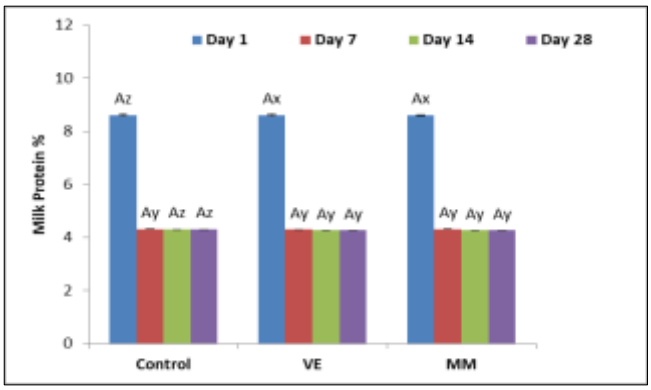

Fig 4: Changes in milk protein (%) in Vitamin E and selenium (VE) and multimineral (MM) supplemented groups as compared to control

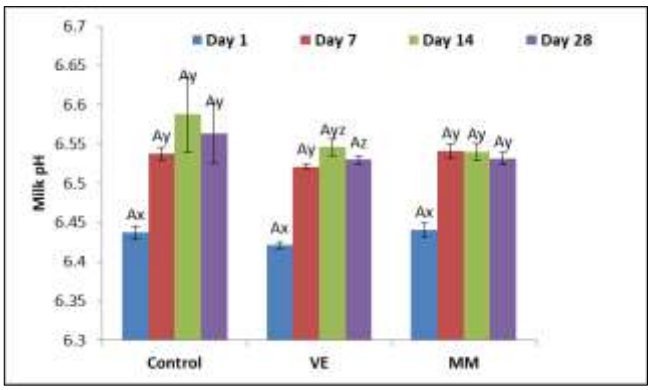

Fig 5: Changes in milk pH in Vitamin E and selenium (VE) and multimineral (MM) supplemented groups as compared to control

Discussion

Vitamin E and multiminerals are reported to be deficient in the body during the periparturient period. The feeding practice varies from farm to farm and from farmer to farmer. Vitamin E is reported to increase the milk production of dairy animals (Singh *et al*., 2021) [28]. Keeping in mind the antioxidative and milk boosting effect of vitamin E and multimineral, strategic administration of these antioxidants was performed to study their effect on the composition of milk during early lactation. The mean fat percentage in the milk of control, VE and MM administered group were 6.350±0.234, 6.934±0.206 and 6.372±0.039 and these findings were in agreement to the values of Murrah buffaloes reported earlier (Ren *et al*., 2015; Arote *et al*., 2021) [23, 2]. The percentage SNF in colostrum and milk observed in this study is higher than reported in earlier studies (Thul *et al*., 2017; Sharma *et al*., 2016; Kumar *et al*., 2015) [30, 27, 16]. SNF value of more than 15 has been reported in colostrum earlier (Bondoc *et al*., 2021) [4]. Such variation in value of SNF is in agreement to study of earlier reports (Arain *et al*., 2008) [1]. The percentage lactose in colostrum was 3.724±0.007, 3.738 ±0.011 and 3.746 ±0.010 for control, VE and MM group respectively and was significantly lower than the lactose content of milk. The percentage of protein in colostrum for control, VE and MM administered groups were 8.611±0.019, 8.606 ±0.023 and 8.603 ±0.023 respectively and were significantly higher than the protein content of milk. The values of protein and lactose for colostrum reported in this study were in agreement to the range of lactose and protein reported for the colostrum of buffaloes by earlier researches (Medhammar *et al*., 2011; Arain *et al*., 2008; Liu *et al*., 2008) [19, 1, 17]. Similarly the range of milk protein and lactose content reported in this study corresponds to the average lactose and protein content reported by previous researchers (Bondoc *et al*., 2021; Medhammar *et al*., 2011; Sarkar *et al*., 2006) [4, 19, 25]. The value of pH reported in this study were in agreement to the range reported earlier (Coroian *et al*., 2015; Han *et al*., 2007) [6].

In the present study, significantly higher (p<0.001) percentage of fat, solid non-fat and protein and significantly lower percentage of lactose was observed in colostrum as compared to milk of buffaloes. Higher fat percentage observed in colostrum in the present study is in agreement to the findings of Singh *et al*., (2021) [28]. Similar variation in SNF, protein and lactose content of colostrum and milk were reported earlier in murrah buffaloes (Bondoc *et al*., 2021) [4]. The higher content of protein in colostrum is accredited to higher concentration of immunoglobulin in colostrum as compared to milk (Bondoc *et al*., 2021) [4]. The significantly higher (p<0.001) SNF content of colostrum in comparison to milk of all groups was in agreement to the finding of earlier research (Bondoc *et al*., 2021) [4]. Coroian *et al*., (2015) [6] reported significant rise in pH of milk on day 7 postpartum and this was in agreement to the finding of this study

The percentage of milk fat on day 1 of calving was significantly higher (p<0.001) in the vitamin E supplemented group as compared to the control group. Similar increase of fat percentage in milk of cows on administration of vitamin E and selenium was reported earlier (Liu *et al*., 2008) [17]. Administration of vitamin E without selenium has also been reported to increase the fat percentage in milk of cows by various researchers (Pottier *et al*., 2006 & Kay *et al* 2005) [21, 14]. Administration of vitamin E in combination with micromineral antioxidants is also reported to significantly (p<0.001) increase the fat percentage of milk of buffalo. Similar increase in fat percentage of buffaloes on administration of Vitamin A was also reported earlier by previous researcher (Yadav *et al*., 2016). This increase in fat percentage on administration of vitamin antioxidant was accredited to improvement in milk synthesis activity of mammary gland (Harmon, 1994) [11].

In the present study no significant (P=0.05) change was observed in the percentage of SNF and lactose of VES and MM groups. Administration of combination of vitamin E and zinc didn't result in any significant change in SNF and lactose content of milk of cows (Chandra *et al*., 2015) [5]. No significant change (P=0.05) in SNF and lactose content of milk on administration of microminerals reported in this study is in agreement to the earlier report (Griffiths *et al*., 2007) [9].

Administration of zinc methionine to cows is also reported to produce any significant change in SNF and lactose content of milk (Kellogg *et al*., 2004) [15]. No significant change (P=0.05) observed in protein content of milk on administration of Vitamin E and selenium and multimineral reported in this study is in agreement to earlier reports in bovine (Chandra *et al*., 2015; Griffiths *et al*., 2007 & Kellogg *et al*., 2004) [5, 9, 15].

Summary & Conclusion

Based on the findings reported in this study, it can be concluded that strategic administration of micronutrient antioxidants during periparturient period can improve milk composition during early lactation.

Ethics statement

All the experimental protocols carried out on laboratory animals were approved by the Sardar Vallabhbhai Patel University of Agriculture & Technology, Modipuram, Meerut- 250110, Uttar Pradesh.

Acknowledgements

Special thanks to Dr. U.K. Dey, Senior Scientist ICAR-Indian Veterinary Research Institute, Bareilly, Uttar Pradesh-India) and other senior authors too. Thanks to the Honorable Director cum vice chancellor, ICAR-IVRI, Bareilly-243122, India and Honorable Vice Chancellor, Sardar Vallabhbhai Patel University of Agriculture & Technology, Modipuram, Meerut- 250110, Uttar Pradesh, India for providing necessary facilities during the tenure of PhD. programme.

Conflict of interest statement

The authors do not have any conflict of interests.

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