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Effect on Milking Performance of Vitamin-Trace Element Supplements to Early Lactation Italian Brown Cows Grazing Ryegrass (*Lolium multiflorum*) Pasture

V. Tufarelli*, R. U. Khan[1] and V. Laudadio
Department of Animal Production, Faculty of Veterinary Medicine,
University of Study of Bari 'Aldo Moro', 70010 Valenzano BA, Italy

ABSTRACT : The objective of this study was to examine the effects of concentrates containing different levels of a vitamin-trace elements premix on milk yield and composition of dairy cows. The trial, which lasted 14 weeks, was conducted from January to March and used 45 multiparous Brown cows in the early phase of lactation. Cows (n = 15 per treatment) were randomly allocated to three dietary treatments: the first group (control, C-0) was fed pelleted concentrate containing background vitamins and trace elements that supplied 1.0 times cows' daily requirements; the second group were fed the same concentrate, but containing 2.5 g/kg of vitamin and trace mineral premix per kg of concentrate (C-2.5); the third group were fed the same concentrate, but containing 5 g/kg of vitamin and trace mineral premix per kg of concentrate (C-5). The daily ration included *ad libitum* chopped oat hay, and the cows also had 8 h/d grazing on a ryegrass (*Lolium multiflorum*) pasture. During the performance trial, cow milk yield was daily recorded and individual milk samples were analysed for milk composition and to determine milk renneting properties. Cows fed the intermediate premix level (C-2.5) in diet showed the highest fat-corrected milk production ($p<0.05$) compared to other groups. None of the milk quality parameters studied were influenced by dietary treatment, except for milk rheological parameters (rennet clotting time and curd firmness) that were positively improved in cows fed the C-2.5 diet ($p<0.05$). The findings from this study show that intermediate level of vitamin-trace elements premix in concentrate can be advantageously used in grazing dairy cows without negative effects on yield and quality of milk produced. (**Key Words :** Cow, Pasture, Vitamin, Trace Element, Milk Yield)

INTRODUCTION

In tropical and subtropical regions dairy cattle usually depend exclusively on native or introduced pastures as their only source of nutrients, and in particular, during critical periods of the year, such as the winter or dry season, the animals cannot fulfil their nutrient requirements because forage is either scarce or of low quality (Soto et al., 2001). Therefore, there is the need to integrate the ration with the optimum level of nutrients to meet grazing animal requirements. There have been numerous studies carried out on the use of dietary vitamins and trace elements, leading to a better understanding of the functions performed by these micro-nutrients, and factors that affect their bioavailability in dairy ruminants (Andrieu, 2008). Moreover, the progress of knowledge has confirmed the beneficial effects of their supplementation in the diet, because these micro-nutrients perform essential and specific roles for the performance of basic physiological processes, and their deficiency or excess adversely affects animal well-being (Spears and Weiss, 2008), fertility (Boland and Lonergan, 2003) and production (Rabiee et al., 2010; Tufarelli et al., 2011).

In recent years there has been an ongoing review of the level of supplementation of vitamins and trace elements for dairy cows on the basis of their production and genetic line (Patton et al., 2006). In fact, the increase of animal products obtained through genetic selection has resulted in animals with significant production performance, requiring a continuous improvement of nutrient intakes. The content of vitamins and trace elements in feed commonly used in ruminants varies greatly depending on different factors such as species, variety and maturity stage of plants, climatic

* Corresponding Author : Vincenzo Tufarelli. Tel: +39-080-5443916, Fax: +39-080-5443811, E-mail: v.tufarelli@veterinaria.uniba.it
[1] Department of Physiology and Pharmacology, University of Agriculture, Faisalabad, Pakistan.
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conditions, season and soil composition (Ramirez et al., 2001). Because of this great variability, it is necessary to use an appropriate program of supplementation. In the intensive farming system special attention should be paid to deficiencies of trace elements and hypo- and hypervitaminosis as these are often difficult to diagnose (Zhang et al., 2010) and that, even in the absence of observable clinical signs, these can affect the animal production and predispose to clinical disease (Ansotegui et al., 1999).

In the preparation of commercial concentrates for different dairy animals it is common practice to include the use of a vitamin-mineral premix. However, in practice the diets for dairy ruminants are often formulated, particularly dairy cows, to contain significantly higher levels of vitamins and minerals than recommended by the National Research Council (NRC, 2001) in order to optimize milk production. To date, the literature lacks studies on the use of vitamin-mineral supplements that confirm how the ingestion of large amounts of vitamins and trace elements lead to improved animal performances and product quality. The aim of this study was to evaluate the effects of adding two different dietary levels of a vitamin-trace elements complex on milk yield and milk quality in grazing dairy cows.

MATERIALS AND METHODS

Animals, diets, and milk trial

The trial was conducted in Bari province, in the Apulia region in Southern Italy (latitude: 41°7′; longitude: 16°52′), for 14 weeks and involved 45 multiparous, early lactating Italian Brown dairy cows. The study included a two weeks adaptation period to the experimental diet, followed by 12 weeks of milk sampling and recording whilst continuing to feed the three experimental diets (from January to March). The cows (632±17 kg body weight; 3.7±0.4 body condition score; 20±6 d in milk at the beginning of the trial) were cared for according observing the animal welfare Directive No. 91/629/EEC (received in Italy by D.L. 533/92 and modified by D.L. 331/98).

Cows were individually housed in box stalls during the adaptation period and in tie-stalls during the sampling period. Each stall had a water trough and a separate manger for hay feeding. Wood shavings were used as the bedding material. Cows had free access to fresh water and did not receive recombinant bovine somatotropin during the whole trial period. Cow health status was checked throughout the study period and no cases of clinical mastitis were recorded. Cows (n = 15 per treatment) were randomly allocated to three dietary treatments according to calving date, parity (2nd or 3rd) and their initial milk yield and composition.

The dietary treatments were as follows: the first group (control, C-0) was fed the pelleted concentrate containing background vitamins and trace elements that supplied 1.0 times the cows' daily requirements; the second group were fed the same concentrate, but containing 2.5 g/kg of vitamin and trace element premix per kg of concentrate (C-2.5); the third group were fed the same concentrate, but containing 5 g/kg of vitamin and trace mineral premix per kg of concentrate (C-5). The ingredient composition of the concentrate and the vitamin/trace element supplement is reported in Table 1. The concentrate contained 18% crude protein and supplied 1.06 milk forage units, both on dry matter basis (Table 2). For all of the treatment groups the daily ration also included *ad libitum* chopped oat hay (Table 2), and the cows also had 8 h/d grazing on natural pasture based mainly on ryegrass (*Lolium multiflorum* Lam) pasture (Table 3). Each cow received the daily amount of hay split into three meals a day, while the concentrate was also offered into three separate equal meals a day (0600, 1200 and 1800 h). The amount of concentrate fed was based on the cow's milk yield, with adjustments of the amount fed being made on a weekly basis. Throughout the trial, each cow consumed the entire concentrate ration they were offered. Individual daily hay intake was determined by subtracting the amount of residue recovered in the manger

Table 1. Ingredient composition of concentrate fed to grazing dairy cows

Item	Concentrate[1]		
	C-0	C-2.5	C-5
Ingredient (g/kg of DM)			
Corn	350	350	350
Soybean meal (44% CP)	150	150	150
Barley	150	150	150
Sunflower meal (28% CP)	87	87	87
Wheat bran	80	77.5	75
Wheat middlings	75	75	75
Dehydrated beet pulp	50	50	50
Calcium carbonate	22	22	22
Dicalcium phosphate	16	16	16
Sodium bicarbonate	10	10	10
Magnesium oxide	5	5	5
Sodium chloride	5	5	5
Vitamin-trace mineral premix[2]	-	2.5	5

[1] C-0 = Concentrate containing no vitamin and trace mineral premix; C-2.5 = Concentrate containing 2.5 g/kg of vitamin and trace mineral premix; C-5 = Concentrate containing 5 g/kg of vitamin and trace mineral premix.

[2] Vitamin and trace mineral premix supplied per kg of product: vitamin A 8×10^6 IU; vitamin D_3 8×10^5 IU; vitamin E 12×10^3 mg; vitamin B_1 2×10^3 mg; D-pantothenic acid 20 mg; choline chloride 5×10^4 mg; vitamin PP 1×10^5 mg; vitamin B_{12} 6 mg; Co 250 mg; Fe 2×10^4 mg; I 1×10^3 mg; Cu 4×10^3 mg mg; Mn 2×10^4 mg Zn 43×10^3 mg; Se 40 mg.

Table 2. Chemical composition (% on DM basis) and calculated analysis of diets fed to grazing dairy cows

Item	Concentrate	Oat hay	SEM
Chemical composition			
DM	87.6	88.4	0.21
Crude protein	18.2	11.6	0.52
Crude fibre	8.6	33.4	0.44
Ether extract	3.2	2.9	0.09
Ash	9.5	11.1	0.23
NDF	20.3	54.5	1.03
ADF	10.3	40.9	0.66
ADL[1]	1.9	7.2	0.11
NFC[2]	48.8	19.9	0.42
Acid insoluble ash	0.6	0.7	0.02
Hemicellulose	10.1	13.6	0.37
Cellulose	7.8	32.9	0.25
Milk FU (*n*/kg DM)[3]	1.06	0.67	-

[1] ADL = Acid detergent lignin.
[2] NFC = 100-(% NDF+% crude protein+% ether extract+% ash).
[3] Milk FU = Milk forage units calculated according to INRA (1989).

Table 3. Chemical composition (% on DM basis) of pasture biomass during the experimental period grazed by dairy cows

Item	Pasture sampling		
	Early period	Late period	SEM
Chemical composition			
Moisture	74.2	71.1	0.85
Organic matter	92.2	95.6	0.91
Crude protein	16.4	15.5	0.52
Crude fibre	25.8	27.3	0.31
Ether extract	1.8	1.2	0.15
Ash	7.7	4.4	0.39
N-free extract	49.4	46.2	0.51
NDF	45.6	48.3	0.75
ADF	32.2	36.4	0.32
ADL	4.4	4.9	0.05
Acid insoluble ash	1.4	1.6	0.03
Hemicellulose	12.2	11.8	0.28
Cellulose	27.5	29.9	0.73
Milk FU (*n*/kg DM) [1]	0.71	0.72	0.02

[1] Milk FU = Milk forage units (INRA, 1989).

from the amount of hay offered to the cow.

The concentrates fed during the trial were formulated to provide similar amounts of crude protein (CP) and meet without exceeding the nutrient requirements (NRC, 2001) of cows yielding 35 kg milk/d with 35 g/kg milk fat and 30 g/kg milk protein. The energy values as milk forage unit (FU) of concentrates and hay were estimated (INRA, 1989).

Body condition score (BCS) of cows was measured by the same observer according to Edmonson et al. (1989). Assessment was based on palpation of the transverse processes of loin vertebrae, cranial coccygeal vertebrae (tail head), and tuber ischii (pin bones). Cows were scored at the beginning and subsequently at the end of trial. Cows were milked twice daily (0600 and 1800 h) using pipeline milking machines.

Feeds and milk analysis

The samples of hay, concentrates, and refusals were collected daily and concentrate feeds were sampled weekly. Samples were ground in a hammer mill fitted with a 1-mm pore size screen and then analysed in triplicate for their dry matter (DM, 65°C in a forced-air oven, dried to a constant weight), ash, CP (N×6.25), crude fiber (CF) and ether extract (EE) contents according to the procedures of the AOAC (2000). Neutral detergent fiber (NDF) acid detergent fiber (ADF) were analyzed according to Van Soest et al. (1991) and was corrected for residual acid insoluble ash (AIA). Acid detergent lignin (ADL) was determined by the method of Van Soest and Robertson (1985). Cellulose and hemicellulose were also estimated as NDF (ash free)-ADF (ash free) and ADF (ash free)-ADL (ash free), respectively. Composite samples were analyzed for AIA as reported by Van Keulen and Young (1977). Non-fiber carbohydrates (NFC) percentages were calculated as 100-(NDF+CP+ether extract+ash).

The daily milk yield was recorded by means of graduated measuring cylinders attached to individual milking units. Individual milk samples, consisting of proportional volumes of morning and evening milk, were taken after cleaning and disinfection of teats and discharging the first streams of foremilk. Samples were collected in 200 ml sterile plastic containers at fortnightly intervals throughout the lactation period and maintained under refrigeration until they were analysed.

The milk samples were analysed for protein content (N×6.38) according to AOAC (2000); fat and lactose contents, using an infrared spectrophotometer (Milko Scan 133B; Foss Electric, DK-3400, Denmark); casein content (AOAC, 2000); as well as a somatic cell count (SCC), using a Foss Electric Fossomatic 90 cell counter. Milk production was standardised and corrected to 4% fat (FCM) according to NRC (2001). Milk pH was measured using a pH-meter with a temperature probe (Expandable Ion Analyzer model EA940 and electrode Ross model 81-02; Research Inc., Boston, MA).

The cheesemaking properties of the milk samples were also determined following three clotting parameters (Zannoni and Annibaldi, 1981) using the Formagraph apparatus (Foss Italia, Padova): rennet clotting time (r), as the time from rennet addition to the beginning of coagulation; curd firming time (k_{20}), as the time from coagulation until reaching the curd firmness corresponding to an amplitude of 20 mm on the Formagraph trace; and curd firmness (a_{30}), as the amplitude of the trace 30 min after the rennet addition. Measurements (in triplicate) were conducted at milk natural pH.

Statistical analysis

Experimental design was a randomized block design, with 15 replicates per treatment. Milk yield and composition parameters of cows were analyzed using the GLM procedure of SAS (2004). Cows within treatment were fitted as term of error. The initial milk yield and composition during the two weeks of dietary adaptation period was introduced into the model as covariate. Data were presented as means of each treatment and standard error of the means (SEM). When significant effect was found, means were compared using Student t-test. Significant differences were stated at $p<0.05$.

RESULTS AND DISCUSSION

The chemical composition of the pasture (Table 3) was in the range expected for annual ryegrass during the winter season in subtropical condition (Meeske et al., 2006; Malleson et al., 2008).

Milk yield and content, as well as cheese-making properties of the milk are reported in Table 4. Supplementation with the vitamin/trace elements had no effect ($p>0.05$) on milk composition or yield, although there was a positive trend ($p = 0.08$) in milk production for cows fed the C-2.5 diet). Kincaid and Socha (2004) also reported a tendency for increased milk production in dairy cows receiving vitamin/trace mineral supplements, as did Siciliano-Jones et al. (2008) when dairy cattle were fed different sources of trace elements. Tufarelli et al. (2011) found supplementation with a vitamin-trace elements complex had a direct, positive effect on milk production of grazing dairy ewes. Likewise, fat and protein production milk were slightly higher ($p = 0.21$ and $p = 0.15$, respectively) for the cows fed the C-2.5 diet than the other dietary groups.

Milk fat-corrected (4%) from cows fed the diet containing the intermediate level of supplement (C-2.5) was higher ($p = 0.04$, Table 4) compared to unsupplemented and C-5 diets (18.3 and 18.1 kg/d, respectively). Figure 1 shows the trend of FCM production over the trial period.

Milk clotting properties in relation to dietary treatments are reported in Table 4. The lack of effect ($p>0.05$) of dietary treatment on curd firming time (k_{20}) may be partly due to the small differences in the milk protein concentration of samples on which the rheological measurements were carried out, as previously observed by Tufarelli et al. (2009). However, in our study the dietary treatment significantly affected the other milk clotting parameters ($p<0.05$) with the milk from dairy cows fed the C-2.5 diet having a better aptitude for coagulation as

Table 4. Effect of dietary treatments on milk yield, composition and clotting properties of grazing dairy cows

Item	Dietary treatment*				
	C-0	C-2.5	C-5	SEM	p-value
Yield [1]					
Milk (kg/d)	19.04	19.64	19.06	0.24	0.08
4% FCM (kg/d) [2]	18.25	18.85	18.08	0.15	0.04
Fat (kg/d)	0.71	0.74	0.70	0.09	0.21
Protein (kg/d)	0.67	0.69	0.66	0.08	0.15
Milk composition					
Fat (%)	3.73	3.78	3.71	0.11	0.07
Protein (%)	3.47	3.48	3.47	0.08	0.28
Lactose (%)	4.91	4.88	4.86	0.04	0.25
pH	6.69	6.72	6.72	0.02	0.27
Milk urea (mg/dl)	26.5	25.2	26.1	0.65	0.07
SCC (cells/ml $\times 10^3$)	395	366	374	158	0.13
Clotting properties [3]					
r (min)	12.79	12.23	12.51	0.22	0.04
K_{20} (min)	2.88	2.44	2.72	0.09	0.16
A_{30}, mm	44.96	49.01	45.13	0.57	0.03

* C-0 = Concentrate containing no vitamin and trace mineral premix; C-2.5 = Concentrate containing 2.5 g/kg of vitamin and trace mineral premix; C-5 = Concentrate containing 5 g/kg of vitamin and trace mineral premix.

[1] Mean for 12 wk of lactation. [2] 4% FCM, fat-corrected milk (%) calculated according to NRC (2001).

[3] r = Rennet clotting time; K_{20} = Curd firming time; A_{30} = Curd firmness.

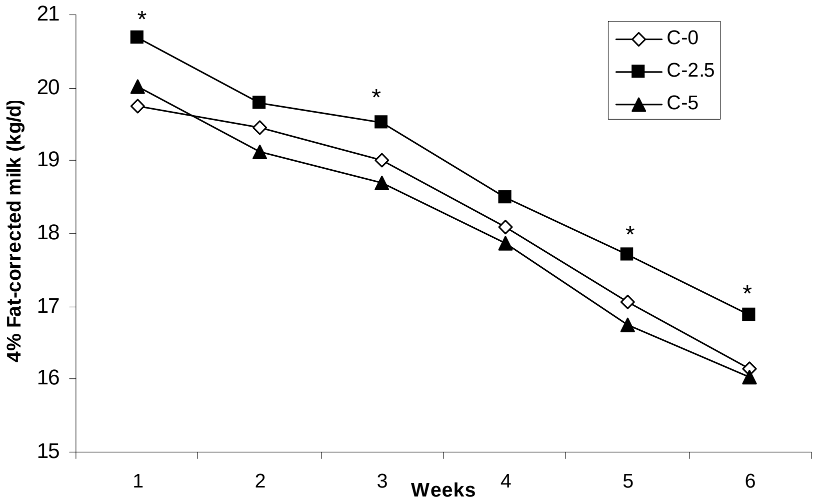

Figure 1. Effect of dietary treatments on 4% fat-corrected milk production (kg/d) of grazing dairy cows. * $p<0.05$.

determined by a low value of the rennet clotting time (r) and a high value of curd firmness (A_{30}). These results could be due to marginally lower SCC and pH values registered in this milk.

Therefore, supplementing dietary vitamin-trace elements complex in concentrate at level of 2.5 g/kg is desirable as it can achieve a major economic return without changes in milking performance of grazing dairy cows.

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