—ORIGINAL—

Effect of β-carotene Supplementation on Italian Trotter Mare Peripartum

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When the mare's estrous cycle resumes in winter, the β-carotene content of hay is depleted. Sixty Italian trotter mares were randomly assigned to a Control or a Treated Group. Treated Group received 1g/d synthetic β-carotene for 15 days from parturition. Blood samples collected at parturition and on days 5, 10 and 15 after partum were analysed for β-carotene, vitamins A, progesterone, 17 β-estradiol, the energy parameters (glucose, cholesterol, NEFA), the protein profile (total protein, albumin, urea) and LDH. Some changes in these measures were attributable to treatment, which significantly affected β-carotene and 17 β-estradiol concentrations. A significant effect was also found on the resumption of estrous activity (χ^2 test=P<0.052).

Key words: *β-carotene, horse, mare, reproduction activity*

J. Equine Sci.
Vol. 21, No. 1
pp. 1–6, 2010

In Italy trotters begin racing in the summer of their second year of life. As a consequence, foals born early in the year have a developmental advantage over those born in late spring-summer. The mare's estrous cycle usually resumes in the spring. The management of trotter mares involves advancing foaling to the early months of the year, thus also advancing the next estrous cycle. An effect of β-carotene supplementation on reproduction has been described in other species [9, 10]. Rasmussen *et al.* [15] documented early on the important role of β-carotene and vitamin A supplements in mares as they studied the plasma provitamin and vitamin A changes induced by a green forage diet. β-carotene and α-tocopherol content in forage was found to be related both to differences in plant species and cultivars and to stage of development at harvest, suggesting that such factors probably affect element content more than the method of conservation [11].

In a study of plasma vitamin A and E in mares and foals Mäenpää *et al.* [13] demonstrated that a diet of hay and oats is low in vitamin A or E, stressing the risk of a vitamin deficit in stabled horses fed hay. Schweigert and Gottwald [16] highlighted the role of β-carotene (a vitamin A precursor) in herbivores for its favourable effect on reproduction; they noted that whereas β-carotene is stored in the equine organism, little is known of liposoluble vitamin content in plasma and milk. They also reported that an increase in plasma β-carotene in the peripartum may promote absorption and/or reduced conversion of provitamin to vitamin A by the mare. β-carotene supplementation decreased days open and services per conception, and reduced the incidence of silent heats in cows [3], as later confirmed by other researchers [6].

Finally examination of different methods of β-carotene administration through fresh forage or synthetic supplements demonstrated that both ensure identical β-carotene bioavailability [8]. β-carotene activity in fodder is impaired even in well-conserved hay. Vitamin A deficiencies are associated with poor fertility in cattle; concentrated feeds contain no β-carotene, while the process of field-curing grass to make hay results in heavy loss of carotene [20]. Oral β-carotene supplementation when mares are not at grass increases pregnancy rates [20].

In addition, the reproductive function is known to be

This article was accepted February 26, 2010
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Table 1. Chemical analysis of the daily ration

	Complete feed	Oats	Hay
Moisture (%)	12.43	13.2	10.30
Crude protein (%)	11.60	13.2	7.53
Crude fibre (%)	8.67	11.4	34.01
Ether extract (%)	3.96	5.7	1.48
ADF (%)	–	–	40.28
NDF (%)	–	–	61.81
ADL (%)	–	–	5.50
Ca (%)	1.09	0.9	0.60
P (%)	0.57	3.8	0.21
β-carotene (mg/kg)	–	–	10.0
DE (MJ/kg)	10.5	11.5	9.01

Table 2. Daily supplies *vs* recommended content according to Martin-Rosset (1990)

	Daily supplies	According to Martin-Rosset*
Dry matter (kg)	9.07	$11 \rightarrow 7.5$
Crude protein (g)	867	$961 \rightarrow 672$
Ca (g)	76	$59 \rightarrow 40$
P (g)	30	$52 \rightarrow 31$
β-carotene (mg)	60 (CG) 1,060 (TG)	
DE UFC	7.5	$8.2 \rightarrow 5.6$

*Higher value recommended for the 1st month of lactation, lower value from the 3rd month onwards.

greatly affected by the diet [12, 19], whose adequacy and efficiency can be monitored by a number of plasma parameters.

The aim of this study was to evaluate the effects of synthetic β-carotene supplementation in the peripartum.

Materials and Methods

Sixty Italian trotter mares aged 5–12 years, live weight at the end of pregnancy 540 ± 14 kg, were studied at a farm in Northern Italy. During the winter (November to April) they were stabled in individual boxes and received a ration containing 6 kg mixed grass hay, 2 kg oats and 2 kg complete feed. Mares were randomly divided into a Control group (CG) and a Treated Group (TG). At parturition and for 15 days Treated Group received a synthetic β-carotene supplement providing 1g/d active principle (Rovimix® β-carotene 10%, kindly provided by Istituto delle Vitamine SpA, Milano, Italy) in addition to 2 kg complete feed, 2 kg oat and 6 kg grass hay.

The study was approved by the Ethics Board of Bologna University, Bologna, Italy.

The chemical composition of feeds [1] is reported in Table 1; in Table 2 the nutritional characteristics of the daily ration are compared to those recommended by Martin-Rosset [14].

All subjects were assigned a BCS of 3 (good body condition) according to Martin-Rossett [14], reflecting the ability of the daily ration to meet their requirements.

Blood collected from the jugular vein at parturition and on days 5, 10 and 15 post partum was placed in vacuum tubes containing Li-heparin. Plasma was obtained by centrifugation (15 min at 3,500 rpm) and immediately frozen (–20°C) for later use to determine:

1. β-carotene, vitamin A (retinol). β-carotene and Vitamin A were extracted from plasma after deproteinization with an equivalent volume of ethanol and n-hexane. Organic extracts were dried, dissolved in ethanol, BHT and THF, and analysed by HPLC;
2. glucose (UV method with hesokinase and glucose-6 phosphate dehydrogenase), cholesterol (method described by Stadtman), NEFA (enzymatic test by the use of acil-CoA synthetase), total protein (biuret

Table 3. Effects of treatment and time of collection on plasma β-carotene and vitamin (mean ± SE)

	β-carotene μg/dl		Retinol μg/dl	
Diet	CG	TG	CG	TG
Subject no.	30	30	30	30
At parturition	13.6 ± 7.4	31.1 ± 6.5	18.5 ± 0.02	22.7 ± 0.03
5 day after partum	7.9 ± 16.9B	71.2 ± 11.6A	21.4 ± 0.03	16.2 ± 0.03
10 day after partum	10.9 ± 13.8B	64.1 ± 8.9A	22.5 ± 0.03	25.0 ± 0.02
15 day after partum	10.0 ± 18.4B	88.5 ± 11.5A	24.7 ± 0.03	30.2 ± 0.03
Treatment (T)	***		ns	
Time of collection (C)	ns		***	
T*C	ns		ns	

***=P<0.0001; ns=not significant.

reaction), albumin (bromocresol method), urea (urease/glutamate dehydrogenase coupled enzymatic technique), and LDH (modified method of enzymatic lactate to pyruvate procedure) were analysed with a DuPont Autoanalyzer at 37°C;

3. progesterone (P_4) and 17 β-estradiol (E_2) were determined by radioimmunoassay techniques [17];

Mares underwent routine obstetrical-gynecological controls to determine the best time for insemination.

Statistical analysis: Data were subjected to analysis of variance with repeated measures by GLM (JMP by SAS) using diet, time of collection and interaction in the model. The following model was adopted:

$$Y_{ij} = \mu + \alpha_i + \beta_j + (\alpha^*\beta)_{ij} + \varepsilon_{ij}$$

where:

Y_{ij} = elements

μ = overall mean

α_i = treatment (C, T)

β_j = time of collection (0, 5, 10, 15 days after partum)

ε_{ij} = experimental error.

The χ^2 test was used to study the differences in the resumption of cyclic activity between groups based on progesterone values.

Results and Discussion

The levels of β-carotene and vitamin A in the four blood samples are reported in Table 3. Significant differences were seen for β-carotene (P<0.0001) due to the diet effect, and vitamin A (P<0.0001) due to the time of collection. In Control mares β-carotene concentrations fell at 5 days, followed by fairly constant levels in the last two samples; in Treated subjects they were significantly higher at parturition and rose rapidly, with a significant effect of treatment, due to the rapid bioavailability of β-carotene, as described by Müller [11]. The means measured in this study were similar to the ones reported by other researchers [9, 16].

Vitamin A levels were similar in both groups in the first two samples, and were significantly higher in Treated mares in the last two. The increase measured after day 5 can be attributed to the availability of the provitamin for transformation into vitamin A. In both groups (CG and TG) the trend of vitamin A was similar to that indicated for the mare postpartum [18].

The metabolic profiles of the two groups are reported in Table 4. Glucose values declined throughout the study, especially in Control Group (P<0.0001). Similar findings have been reported by Trombetta *et al.* [19] at parturition. Cholesterol diminished significantly (P<0.01) in both groups and was higher than the values reported by Trombetta and co-workers [19]. The means measured at parturition were similar to those described by Kienzle *et al.* [8], whereas the trends of the various parameters were similar to those found by Mäenpää *et al.* [13] in mares receiving vitamin A, D, and E supplements. In contrast, NEFA displayed a non-linear behaviour, with significant differences due to the time of collection (P<0.05) being found in both groups. Also in the study by Kawashima *et al.*[6] cholesterol and NEFA did not differ between the groups.

A decrease 5 days after parturition, followed by an increase, was documented in the two farms studied by Trombetta *et al.* [19].

The protein and enzyme profiles (Table 5) showed

Table 4. Effects of treatment and time of collection on energy parameters (mean ± SE)

	Glucose mmol/*l*		Cholesterol mmol/*l*		NEFA μEq/*l*	
Diet	CG	TG	CG	TG	CG	TG
Subject no.	30	30	30	30	30	30
At parturition	6.1 ± 0.54	6.0 ± 0.48	2.4 ± 0.14	2.4 ± 0.12	214.9 ± 4.3	189.2 ± 42.1
5 day after partum	5.1 ± 0.22	4.9 ± 0.19	2.2 ± 0.22	2.2 ± 0.09	109.8 ± 29.1	107.2 ± 25.9
10 day after partum	4.5 ± 0.30	4.8 ± 0.27	2.1 ± 0.07	2.0 ± 0.06	178.5 ± 38.6	147.2 ± 34.3
15 day after partum	3.7 ± 0.25	5.0 ± 0.22	2.1 ± 0.07	2.0 ± 0.06	123.9 ± 33.5	114.0 ± 29.8
Treatment (T)	ns		ns		ns	
Time of collection (C)	***		**		*	
T*C	ns		ns		ns	

*=P<0.05; **=P<0.001; ***=P<0.0001; ns=not significant.

Table 5. Effects of treatment and time of collection on protein and enzyme profiles (mean ± SE)

	Total protein g/d*l*		Albumin g/d*l*		Urea mmol/*l*		LDH UI/*l*	
Diet	CG	TG	CG	TG	CG	TG	CG	TG
Subject no.	30	30	30	30	30	30	30	30
At parturition	7.0 ± 0.14	6.7 ± 0.12	3.3 ± 0.10	3.3 ± 0.09	6.6 ± 0.32	5.5 ± 0.28	322.3 ± 15.9	266.5 ± 14.1
5 day after partum	6.9 ± 0.13	6.9 ± 0.12	3.3 ± 0.08	3.3 ± 0.07	6.4 ± 0.24	5.6 ± 0.22	263.2 ± 13.6	275.9 ± 12.05
10 day after partum	6.9 ± 0.19	6.7 ± 0.17	3.4 ± 0.07	3.2 ± 0.06	6.9 ± 0.22	6.4 ± 0.23	250.8 ± 14.6	240.0 ± 13.0
15 day after partum	6.8 ± 0.15	6.7 ± 0.13	3.3 ± 0.07	3.1 ± 0.06	6.9 ± 0.25	6.8 ± 0.22	239.5 ± 13.8	244.7 ± 12.27
Treatment (T)	ns		ns		***		ns	
Time of collection (C)	ns		ns		***		**	
T*C	ns		ns		ns		*	

*=P<0.05; **=P<0.001; ***=P<0.0001; ns=not significant.

significant differences due to treatment only for urea and time of collection for urea and LDH. The total protein means were analogous and consistent in both groups, and were similar to those reported by Mäenpää *et al.* [12] in mares in February and April and in mares receiving vitamin A, D and E supplements [13].

Albumin concentrations were constant over time and were similar to the means measured in growing ponies receiving a daily supplement of 12 μg/kg/d vitamin A [4].

A lower urea concentration was found in the first sample in Treated Group, but it approached the values found in CG already on 10 days after partum. These data are comparable to those reported by Trombetta *et al.* [19] at parturition.

Only LDH showed significant differences for time of collection (P<0.001) and a significant T*C interaction (P<0.05), with varying and different values in the two groups. Our values are lower than those described in growing ponies receiving different vitamin A supplements [4].

Analysis of the hormonal parameters (Table 6) showed significant differences due to the time of collection for both hormones; only E_2 was significantly influenced by treatment.

P_4 increased in the last blood sample (15 days after partum) in cyclic mares and remained low in non-cyclic ones. Analysis of individual progesterone levels showed different trends in the two groups. Five days after parturition they rose in 20/30 (67%) TG and in 12/30 (40%) CG, reflecting the resumption of cycling. Such difference was significant (χ^2 test=P<0.052) and indicates a favourable effect of β-carotene on reproduction activity. A similar effect was also found by Kawashima *et al.* [6], who suggested that β-carotene supplementation may induce better reproductive performances by reducing the time to the next ovulation, but not by Watson *et al.* [20].

The higher E_2 levels found in Treated group can be attributed to a greater availability of retinoic acid for

Table 6. Effect of treatment and time of collection on the reproductive hormones (mean ± SE)

	Progesterone ng/m*l*		17β-estradiol pg/m*l*	
Diet	CG	TG	CG	TG
Subject no.	30	30	30	30
At parturition	7.2 ± 1.022	9.8 ± 0.887	10.3 ± 2.01	19.3 ± 2.2
5 day after partum	0.9 ± 1.022	1.4 ± 0.987	4.7 ± 2.02	6.2 ± 1.1
10 day after partum	2.9 ± 1.022	1.6 ± 0.901	8.9 ± 2.02	8.3 ± 1.5
15 day after partum	8.3 ± 0.987	6.6 ± 0.901	4.2 ± 2.02	4.9 ± 1.6
Treatment (T)	ns		*	
Time of collection (TC)	**		**	
T*C	ns		ns	

*=P<0.05; **=P<0.001; ns=not significant.

the ovaries [9]. Plasma P_4 and E_2 were similar to those reported by Falaschini *et al.* [5]. The interpartum interval was shorter in TG, albeit not significantly so (369.3 *vs* 375.1 days).

Our findings enable some conclusions to be drawn. Synthetic β-carotene induced changes in plasma levels of provitamin and vitamins A, with favourable metabolic effects; however, any positive effects on reproductive efficiency may have been hidden by the short duration of the study. A study involving longer supplementation (30 days) with different β-carotene sources (dehydrated alfalfa and synthetic β-carotene) is under way to confirm these data.

Acknowledgments

The study was supported by a grant of Alma Mater Studiorum University of Bologna, Bologna, Italy (Project Manager A. Falaschini).

We are grateful to Istituto delle Vitamine S.p.A., Milano, Italy, for providing ROVIMIX® β-carotene 10%, and to Orsi Mangelli Stud, San Giovanni in Persiceto, Italy, for technical and management support.

The paper must be attributed equally to the authors.

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