BMC Veterinary Research

RESEARCH Open Access

Effects of maternal dietary supplementation with antioxidants on clinical status of mares and their foal

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Abstract

Background The peripartum period constitutes a delicate physiological moment in mares showing a transient state of oxidative stress. Diet supplementation with antioxidants during pregnancy in women appears to have a beneficial effect on mother and neonate health. The aim of this work was to evaluate the effects of diet supplementation with a commercial product containing a mix of antioxidants (Oxyliver®, Candioli) on the length of gestation, weight, and haemato-biochemical parameters in Italian Salernitano mares and their newborn foals. Eight late-term pregnant mares were randomly divided into two groups: Antiox group receiving 30 g/day of antioxidants, and Car group receiving the same amount of carrot powder, from 290 to 320 days of gestation. The following parameters were evaluated in mares: weight, colostrum composition, haemato-biochemical parameters, progesterone, and cortisol blood concentrations, along with blood oxidant/antioxidant *status*. Assessments were conducted at specific time points: immediately before the start of diet supplementation (T0), 15 days after (T1), at the end of diet supplementation (T2), within 8 h after parturition (T3), and 10 days post-partum (T4). Foal parameters such as weight, haemato-biochemical values, cortisol concentration, and blood oxidative stress variables were assessed within 8 h of birth (TF0) and at 10 days of age (TF1).

Results Pregnancy was shorter in the Antiox group ($P < 0.05$) compared with the Car group; the foals' weight increase of group Antiox (40%) was higher ($P < 0.05$) compared to those of the Car group (28.6%). The colostrum of the Antiox group exhibited higher levels of Brix, total solids, protein, nonfat solids, casein, urea, density, free fatty acids, and glucose, while lower levels of fat and lactose were observed compared to the Car group ($P < 0.05$). Mares' serum albumin at T1 and T3, creatinine, glucose, total proteins, total bilirubin, AST, and ALT at T3 were lower in Antiox than in the Car group. No significant differences were found in foals.

Conclusions While the limited sample size and the potential variability of evaluated parameters, the observed outcomes suggest that Oxyliver® supplementation in mares might safely decrease gestation length and enhance liver function, thus potentially improving colostrum quality and offspring development.

Keywords Mare nutrition, Colostrum, Oxidative stress, Newborn foals, Pregnant mares, Liver function

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Background

Pregnancy and parturition are identified as physiological conditions that could be responsible for redox potential imbalance in mares [1–3]. The peripartum period is the most critical phase in terms of oxidative stress [1–3]. Indeed, the increasing nutrient requirements of the fetus in the final weeks of pregnancy, along with parturition changes in metabolic and endocrine functions, results in an elevation of reactive oxygen species (ROS) [4, 5]. During pregnancy, ROS are mainly produced by placenta and play a dual role, with both adverse destructive functions and beneficial regulatory effects in the normal replacement and metabolism of the placenta [6]. An excess of ROS, named oxidative stress, initiates a pro-inflammatory process, which can result in functional and structural damage to cells, ultimately leading to dysfunctions in the placenta [7, 8]. A connection between placental dysfunction in the late stages of fetal development and low birth weight and immaturity in foals has been reported [2].

The nutrition of a pregnant mare influences the growth of the fetus and the health of the offspring, potentially affecting the risk of developing certain neonatal diseases [9, 10]. Appropriate nutrition, particularly with vitamins, during peripartum period is recommended in mares by NRC [11, 12]. Evidence of the benefits of dietary antioxidant supplementation with vitamins E, C, and β-carotene in horses' diets under stressful conditions has been documented [13]. Recently, dietary supplementation with some antioxidants (β-carotene, α-tocopherol and selenium) has been tested in late pregnant mares, reporting effects on gestation, lactation, neonatal features and on fertility [14–19]. Positive effects of oral supplementation of antioxidants during the last quarter of pregnancy in mares have been reported on placental efficiency, neonatal weight, oxidative stress status and gestation length [14–20]. Moreover, β-carotene supplementation to late pregnant mares increased colostral immunoglobulins, antioxidant concentrations in plasma, colostrum and milk of mares and plasma of their foals [15, 16]. Dietary supplementation of prepartum mares with vitamin E and selenium maintained high vitamin E serum concentrations and increased postpartum fertility [14]. Although some antioxidant substances have already been tested in horses, few data are available on the use of antioxidant mixes in late pregnant mares, particularly those that are commercially available. The Salernitano is an Italian warmblood breed native to Southern Italy, specifically in the Campania Region. This very ancient breed is currently considered endangered, and the Regional Institute for the Improvement of Equine Breeds in the Campania region (*Centro Regionale di Incremento Ippico di Santa Maria Capua Vetere*; CRII) instituted a breeding plan to increase their horse population [21].

This study aims to compare the effects of oral administration of two supplements (commercially available mix of antioxidants vs. carrot powder) in mares in the last month of pregnancy, on the length of gestation, colostrum compositions, maternal and neonatal weight, and haemato-biochemical parameters, measuring the changes in blood levels of cortisol, progesterone, and oxidant/antioxidant status.

Results

In the present study, no concentrate and supplement refusals were observed during the diet supplementation of both groups, while the hay refusals were recorded daily to calculate the feed intake (Table 1). No statistical differences of daily intake were observed between groups and time points. All mares completed the study and no complications or side effects related to the diet supplementation were observed. Gestation length was shorter ($P<0.05$) in mares of the Antiox group (321.5 (319.5–322) d) than those of the Car group (334 (333–338) d). Each mare of both groups delivered viable foals and shed a normal and intact placenta spontaneously. No pregnancy or parturition abnormalities were detected up to 10 days *post-partum*. Mares in the two groups did not differ in age or parity. Three male and one female foal were born from the mares of the Car group, while two male and two female foals were born from the Antiox group, with no statistical difference between groups ($P>0.05$). Due to reasons related to the field situation, blood samples and body weight measurements were not collected from all the patients at each time point. However, despite the small number of observations, statistically significant differences were observed between groups.

Body condition score (BCS) and weight

All subjects presented an ideal BCS (3-3.5/5 points) for mares during late pregnancy [22], and no differences were found between groups ($P>0.05$). No differences were found in body weight between groups at each time point or between time points within each group (Table 1). Weight loss (%) of mares between T2-T3 (−9.2 (-11 to -9)% vs. -7.2 (-10 to -6.1)% T2 weight in Antiox and Car group, respectively) and T3-T4 (-1.8 (-2 to -0.9)% vs. 0 (-4,4 to -0.8)% T3 weight in Antiox and Car group, respectively) were not different between the two groups. Foal weight at parturition (TF0) did not differ between groups, being 50 (45–50) kg in the Antiox group vs. 60 (50–65) kg in the Car group. Meanwhile, the weight increase (%) of foals was higher *(*$P<0.05$) in the Antiox group (median (IQR); 40 (40- 57.5) % of birth weight) than in the Car group (28.6 (21.8–30.9)% of birth weight).

Table 1 Trend of Mare body weight (BW) and feed intake

Group	Time points									
	T0		T1		T2		T3		T4	
	Car ($n=4$)	Antiox ($n=4$)	Car ($n=4$)	Antiox ($n=3$)	Car ($n=4$)	Antiox ($n=3$)	Car ($n=3$)	Antiox ($n=3$)	Car ($n=3$)	Antiox ($n=3$)
Mare BW (kg)	585 (550–620)	550 (535–575)	605 (580–630)	550 (545–585)	580 (555–645)	530 (525–535)	620 (595–630)	510 (500–525)	620 (570–635)	510 (495–520)
Feed intake (kg/d)	10.53 (9.90-11.16)	9.90 (9.63–10.35)	10.89 (10.44-11-34)	9.90 (9.81–10.53)	10.44 (9.99–11.61)	9.54 (9.45–9.63)	11.16 (10.71–11.34)	9.18 (9.00-9.45)	11.16 (10.26–11.43)	9.18 (8.91–9.36)
Pelletted concentrate Intake (Kg/d)	2.34 (2.20-2.48)	2.21 (2.14–2.30)	2.42 (2.32–2.52)	2.24 (2.18–2.52)	2.73 (2.22–2.58)	2.12 (2.10–2.14)	2,47 (2.38–2.52)	2.05 (2.00-2.11)	2.43 (2.28–2.54)	2.03 (1.99–2.10)

Table 2 Quality and chemical composition of colostrum of mares of Car and Antiox groups

Colostrum analysis	Car ($n=4$)	Antiox ($n=4$)
Brix (%)	23* (21.5–25.5)	27 (27.0-28.5)
Fat (%)	2.58** (2.58–2.58)	1.39 (1.38–1.40)
Protein (%)	12.0** (12.0–12.0)	15.5 (15.2–15.7)
Lactose (%)	2.19** (2.19–2.19)	1.32 (1.27–1.38)
Total solids (%)	20.1* (20.1–20.1)	22.1 (21.8–22.3)
Solids nonfat (%)	16.3** (16.3–16.2)	19.4 (19.1–19.6)
Casein (%)	9.0** (9.0–9.0)	11.7 (11.5–11.9)
Urea (mg/dL)	20.1** (20.0-20.2)	55.1 (54.9–55.3)
Density (g/L)	1052** (1052–1052)	1065 (1064–1065)
Free fatty acid (meq)	0.42** (0.42–0.42)	0.46 (0.46–0.46)
Glucose (%)	0.74** (0.74–0.74)	0.90 (0.88–0.91)

Values are reported as median and interquartile range (IQR)

*, ** indicate a significant difference between groups ($p<0.05$ and $p<0.001$, respectively)

Colostrum analysis

The quality and chemical composition of colostrum of mares of both groups are reported in Table 2. The Brix value and the percentage of total solids were higher ($P<0.05$) in the Antiox group than in the Car group. Higher ($P<0.001$) levels of proteins, solids nonfat, casein, urea, density, free fatty acid, and glucose were found in the Antiox group than in the Car group. Otherwise, the Antiox groups showed lower ($P<0.001$) values of fat than the Car group.

Haemato-biochemical parameters

Haemato-biochemical results of mares and foals included in this study at each time point are reported in Tables 3 and 4, and 5. No differences in blood count parameters were found between groups or time points in both mares and foals (Tables 3 and 5). Differences between groups in biochemical values were found in mares, revealing lower ($P<0.05$) values of albumin at T1 and T3, creatinine, glucose, TP, total bilirubin, AST, and ALP at T3 in the Antiox group compared to the Car group. There were no significant differences in any biochemical values of foals between groups or time points (Table 5).

Table 3 Blood count results of mares of Car and Antiox groups at scheduled time points

Mares	**Time points**									
Blood count results	**T0**		**T1**		**T2**		**T3**		**T4**	
Group	**Car (*n* = 4)**	**Antiox (*n* = 4)**	**Car (*n* = 4)**	**Antiox (*n* = 3)**	**Car (*n* = 4)**	**Antiox (*n* = 3)**	**Car (*n* = 3)**	**Antiox (*n* = 3)**	**Car (*n* = 3)**	**Antiox (*n* = 3)**
Erythrocytes	8.3	8.5	8.8	8.2	8.7	7.5	7.9	7.5	8.7	7.4
(M/ µL)	(8.3–8.6)	(8.3–8.8)	(8.6-9)	(7.5–8.3)	(8.4–8.8)	(7.3–7.6)	(7.6–8.7)	(7.3–7.7)	(8.5–8.7)	(7.3–7.5)
Hematocrit	37.9	39.3	41.3	39	40.9	34.6	38.2	36	40.3	33.8
(%)	(37.8–40.5)	(38.1–40.9)	(40.2–42)	(34.5–39.5)	(39.7–41.6)	(33.8–35.3)	(35.8–41.4)	(33.9–36.9)	(39.6–40.6)	(32.8–34.7)
Hemoglobin	13.8	14.1	14.9	14.1	14.1	12.5	13.5	12.8	14.4	12.2
(g/ dL)	(13.4-14-4)	(13.6–14.8)	(14.4–15.2)	(12.5–14.3)	(13.3–14.8)	(12.2–12.9)	(12.8–14.6)	(12.1–13.2)	(14.2–14.4)	(11.9–12.6)
Leucocytes	9.3	10.8	10.2	11.2	9.7	9.4	11.2	12.2	11.2	11.6
(K/ µL)	(8.5–10.5)	(10-11.3)	(9.8–10.7)	(10.5–11.5)	(9.2–10.1)	(8.7–10.1)	(10.7–11.6)	(11.9–12.1)	(10.3–12)	(11.2–11.9)
Neutrophils	4.9	5.7	5.1	5.7	5.5	6.3	7.5	8.8	7.8	6.8
(K/ µL)	(4.8–5.4)	(5.6-6)	(4.9–5.5)	(5.7–6.3)	(4.8–6.1)	(6.1–6.6)	(7.3–8.2)	(8.4-9)	(7.1–7.8)	(6.8–6.8)
Lymphocytes	3.8	3.9	4.4	5	3.6	3	2.7	2.9	2.9	3.9
(K/ µL)	(2.7–4.4)	(3.4–4.6)	(4-4.5)	(3.6–5.1)	(2.8–4.3)	(2.2–3.7)	(2.2–3.3)	(2.9–3.5)	(2.8–3.3)	(3.6–4.2)
Monocytes	0.43	0.40	0.42	0.49	0.42	0.41	0.28	0.47	0.35	0.38
(K/ µL)	(0.40–0.43)	(0.33–0.49)	(0.41–0.46)	(0.45–0.50)	(0.38–0.44)	(0.38–0.44)	(0.26–0.37)	(0.45–0.61)	(0.35–0.42)	(0.34–0.42)
Eosinophils	0.14	0.19	0.27	0.14	0.20	0.11	0.08	0.06	0.21	0.42
(K/ µL)	(0.11–0.18)	(0.15–0.25)	(0.20–0.36)	(0.14–0.16)	(0.17–0.21)	(0.09–0.12)	(0.08–0.14)	(0.05–0.1)	(0.15–0.43)	(0.41–0.44)
Basophils	0.08	0.04	0.05	0.03	0.03	0.04	0.04	0.03	0.02	0.07
(K/ µL)	(0.03–0.08)	(0.02–0.06)	(0.04–0.05)	(0.03–0.04)	(0.02–0.04)	(0.04–0.04)	(0.03–0.07)	(0.03–0.04)	(0.02–0.02)	(0.04–0.09)
Platelets	97	130	122	123	113	128	132	120	119	124.5
(K/ µL)	(80–115)	(118-147.3)	(90.8–143)	(108-125.5)	(101.5–121)	(127.5-128.5)	(88.5-135.5)	(107.5–135)	(85–136)	(120.8-128.3)

T0: before the start of diet supplementation; T1: 15 days of diet supplementation; T2: at the end of diet supplementation; T3: within 8 h of foaling; T4: 10 days post-partum; values are reported as median and interquartile range (IQR)

Progesterone, cortisol, d-ROMs and BAP blood concentrations

The results of progesterone, cortisol, d-ROMs, and BAP blood levels in mares at each time point are exhibited in Fig. 1. No differences between groups or time points were found, except for BAP concentration at T3, which was higher in mares of the Car group compared with mares of the Antiox group ($P<0.05$, Fig. 1D). No differences were found between groups and time points for cortisol, d-ROMs, and BAP blood concentrations in foals, as reported in Table 5.

Discussion

Despite the evidence of oxidative stress occurring during late pregnancy in mares [1–3], there are limited number of reports on the effects of dietary supplementation with a mix of antioxidants on mares and their foals' health [14–19]. The present study evaluated for the first time the effects of oral supplementation with a specific commercial mix of antioxidants formulated to support liver function (Oxyliver®) in late pregnant-mare on the length of gestation, hemato-biochemical parameters, and newborn foal development, monitoring the changes in progesterone and cortisol concentrations and oxidant/antioxidant status in the blood of both mares and foals. The Oxyliver® supplementation was compared with the supplementation in equal dosage of carrot powder.

Nutritional supplementation started at 290 days of gestation to ensure a minimum of 30 days of dietary treatment in all mares, considering the mares physiological gestation length (320–360 days) [23]. This specific study period was chosen to investigate and support the transient redox potential imbalance observed immediately *post-partum* in mare, that is also responsible for the neonatal health [1–3]. Other studies with a similar experimental design (evaluation period and stage of pregnancy) have achieved beneficial effects from diet supplementation with antioxidants (e.g. tocopherols) [17, 18, 20]. Mares in late pregnancy, when the fetus is rapidly growing, have specific nutritional requirement [22] and it is necessary to increase the amount of concentrate [24]. Moreover, considering the low quality of hay observed in the last years in Southern Italy [25], particularly in vitamin contents, a specific micronutrients supplementation is necessary to compensate the lack of vitamins. In this regard, the addition of Oxyliver® to standard diet increased the vitamins content. In particular the vitamin E intake observed in Antiox group guaranteed the satisfaction of vitamin requirement, for pregnant mares [11, 12]. Two different studies [20 e 26] observed that a daily vitamin intake of 2500 IU was able to increase the alpha-tocopherol and immunoglobulins concentrations in colostrum and milk and the foal's serum. Although synthetic vitamin E, found in concentrates and supplements, is less effective at raising blood levels of α-tocopherol compared to natural vitamin E, it can still bind to the tocopherol transport protein, facilitating its transfer from the liver into circulation [26]. This process helps interrupt free radical chain reactions, thereby contributing to the prevention of oxidative stress-related damage [26].

Several authors reported that the combined administration of different antioxidants is preferred over the use of a single antioxidant because a mix of antioxidants acts in synergy, potentiating their effects [27, 28]. This specific commercial mix of antioxidants, not specifically formulated to support pregnancy, was chosen to compare the antioxidant efficiency with β-carotenes, used as positive control. Both used supplements were dosed in accordance with manufacturer recommendations. In addition, the use of products already on the market gave the opportunity to safely test antioxidants in pregnant mares, minimizing the risk of unexpected adverse effects.

Notwithstanding the small sample size, the administration of two different antioxidants was associated to significant differences in mares and foals clinical parameters. The results of this study demonstrated that 30 days of supplemental feeding with the commercial mixture of antioxidants in late-pregnant mare safely shortened pregnancy length. Although the canonical range for equine is reported to be between 320 and 360 days, physiological gestation length have been only recently reported in Salernitano mares; all mares included in this study foaled within those ranges (313 to 350 days) [29, 30].Various intrinsic and extrinsic factors, including nutrition, breed, age, and parity of the mares, as well as the gender of fetus, have been implicated in influencing pregnancy duration [31–33]. In this study, only mares of the same breed were included, and no differences were found for age, parity, season, and foal gender. Similar results were observed after diet supplementation of pregnant mares with selenium for 110 days before foaling [16] and with a specific amino acid inducer of antioxidant response (L-arginine) [34] for 21 days before foaling [35]. Mortensen et al., [35] hypothesized that supplementation would initiate parturition by increasing fetal adrenal function, resulting in elevated cortisol levels and a subsequent decrease in progesterone levels in mares [35]. In this study, no differences in progesterone and cortisol concentrations were observed in mares during all trial, suggesting that in our case the reduction of pregnancy length was not due to these hormones.

The period of gestation and duration of supplementation are additional factors to consider when attempting to understand the mechanism of action of antioxidants, which remains not fully elucidated. Contrasting results have indeed been reported regarding the influence of the length and period of antioxidant dietary intake on reproductive performances in sows, whose placenta is similar

Table 4 Biochemical results of mares of Car and Antiox groups at scheduled time points

	Time points									
Biochemical parameters	**T0**		**T1**		**T2**		**T3**		**T4**	
Group	**Car (*n* = 4)**	**Antiox (*n* = 4)**	**Car (*n* = 4)**	**Antiox (*n* = 3)**	**Car (*n* = 4)**	**Antiox (*n* = 3)**	**Car (*n* = 3)**	**Antiox (*n* = 3)**	**Car (*n* = 3)**	**Antiox (*n* = 3)**
Urea (mg/dL)	17 (17–21)	23 (18.5–28)	21 (19.5–22)	20 (16.5–23)	37.5 (32.8–42.3)	37.5 (32.8–42.3)	23 (19-28.5)	17 (16–21)	15 (14.5–18.5)	21 (19.5–21)
Creatinine (mg/dL)	1.2 (1.2–1.4)	1.6 (1.5–1.7)	1.1 (1.1–1.3)	1.1 (1.1–1.1)	1.4 (1.3–1.5)	1.2 (1.1–1.3)	1.5 (1.3–1.7)	1.1* (1.0-1.1)	1.2 (1.2–1.3)	1.3 (1.1–1.4)
Glucose (mg/dL)	88 (73–92)	80 (75–86)	80 (72–90)	99 (75–105)	71 (67–77)	107 (102–111)	85 (85–93)	72* (64–78)	60 (53–80)	77 (66–112)
TP (g/dL)	5.4 (5.0-5.8)	5.9 (5.9-6.0)	5.4 (5.0-5.8)	4.0 (3.9–4.5)	4.9 (4.8–5.1)	5.7 (5.4–6.1)	6.2 (6.1–6.7)	4.5* (4.3–4.8)	5.8 (5.8-6.0)	5.8 (5.4-6.0)
Albumin (g/dL)	2.5 (2.0-2.9)	3.0 (2.9-3.0)	2.5 (2.4–2.6)	2.0* (1.9-2.0)	2.2 (2.1–2.3)	2.7 (2.6–2.8)	2.9 (2.9–3.1)	2.2* (2.2–2.3)	2.6 (2.6–2.9)	3.0 (2.7–3.1)
Total bilirubin (mg/dL)	0.9 (0.9–1.3)	1.4 (1.2–1.7)	0.9 (0.6–1.4)	0.8 (0.7–1.1)	1.3 (1.0-1.5)	1.0 (0.9–1.1)	2.3 (1.8–2.5)	0.8* (0.8–0.9)	1.5 (1.4–1.8)	0.5 (0.5-1.0)
AST (mg/dL)	173 (135–182)	192 (170–224)	174 (159–207)	103 (101–135)	138 (116–204)	198 (174–222)	238 (210–238)	118* (111–134)	224 (193–225)	232 (189–236)
ALP (mg/dL)	2.0 (1.0–2.0)	1.5 (1.0-2.3)	2.5 (2.0-3.8)	3.0 (2.5-3.0)	2.0 (1.8–3.5)	3.5 (3.3–3.8)	5.0 (4.5-6.0)	1.0* (1.0-1.5)	3.0 (3.0-3.5)	5.0 (4.5-5.0)
GGT (mg/dL)	11.6 (11.3–13.0)	14.8 (12.6–16.8)	13.1 (10.7–19.1)	7.6 (7.6–12.0)	9.6 (8.9–10.3)	15.8 (13.1–18.5)	16.5 (13.1–24.0)	10.1 (8.3–11.3)	15.6 (12.5–22.8)	13.2 (12.3–16.7)
AP (U/l)	292 (217–362)	348 (296–438)	241 (227–367)	237 (201–351)	255 (248–270)	564 (450–677)	447 (406–697)	487 (408–522)	396 (388–606)	489 (456–578)

TP: total proteins; AST: aspartate aminotransferase; ALP: alanine aminotransferase; GGT: gamma-glutamyl transferase; AP: alkaline phosphatase. T0: before the start of diet supplementation; T1: 15 days of diet supplementation; T2: at the end of diet supplementation; T3: within 8 h of foaling; T4: 10 days post-partum

Values are reported as median and interquartile range (IQR)

* indicates a significant difference between groups ($p < 0.05$)

Table 5 Blood parameters of foals born from mares of Car and Antiox groups

Foal	Time points			
Parameters	**FT0**		**FT1**	
Group	**Car (*n* = 4)**	**Antiox (*n* = 4)**	**Car (*n* = 4)**	**Antiox (*n* = 4)**
Erythrocytes (M/ µL)	11.1 (11.1–11.4)	11.7 (11.6–12.3)	9.2 (8.6–9.8)	9.21 (9.0-9.5)
Hematocrit (%)	47.5 (46.8–47.5)	48.9 (48.1–49.3)	33.4 (32.7–37.2)	32.3 (31.9–33.2)
Hemoglobin (g/ dL)	15.6 (15.3–15.9)	15.9 (15.8–16.1)	12.2 (11.9–13.5)	11.7 (11.5–12.1)
Leucocytes (K/ µL)	10.8 (10.1–11.8)	9.5 (8.8–10.9)	12.0 (11.3–12.4)	10.5 (9.6–11.4)
Neutrophils (K/ µL)	8.7 (8.1-9.0)	6.5 (6.1–7.9)	8.3 (7.5–8.8)	5.3 (5.1–6.6)
Lymphocytes (K/ µL)	1.8 (1.6–2.4)	2.1 (1.9–2.5)	3.0 (2.8–3.2)	3.7 (3.6-4.0)
Monocytes (K/ µL)	0.28 (0.26–0.30)	0.49 (0.33–0.62)	0.65 (0.52–0.70)	0.65 (0.57–0.70)
Eosinophils (K/ µL)	0 (0–0)	0 (0–0)	0.02 (0.2 – 0.03)	0.05 (0.04–0.06)
Basophils (K/ µL)	0.07 (0.04–0.09)	0.05 (0.03–0.06)	0.04 (0.04–0.05)	0.03 (0.03–0.04)
Platelets (K/ µL)	185 (164-230.5)	204 (196–269)	138 (97–152)	197 (162–215)
Urea (mg/dL)	22 (16.5–23.5)	23 (22–29)	8.5 (7.3–9.8)	7 (7–7)
Creatinine (mg/dL)	1.2 (1.2–1.7)	1.51 (1.4–1.5)	0.9 (0.9–1.9)	0.9 (0.9–0.9)
Glucose (mg/dL)	137 (95–151)	114 (83–137)	122 (100–144)	134 (117–154)
TP (g/dL)	4.4 (4.0-4.5)	4.8 (4.2–5.3)	4.4 (4.2–4.6)	3.9 (3.9–4.8)
Albumin (g/dL)	2.3 (2.3–2.4)	3.1 (2.7–3.3)	2.2 (2.0-2.3)	2.1 (2.1–2.4)
Total bilirubin (mg/dL)	3.8 (2.1–4.1)	2.8 (2.1–5.1)	1.6 (1.6–1.7)	1.1 (1.1–1.2)
AST (mg/dL)	100 (72–129)	78 (65–115)	361 (298–424)	325 (255–339)
ALP (mg/dL)	6.0 (4.0–7.0)	6.0 (4.0–7.0)	3.5 (2.3–4.5)	8.0 (6.5–13.5)
GGT (mg/dL)	44.9 (28.1–46.1)	19.2 (18.4–21.6)	90.1 (60.5-119.6)	80.4 (56.2–87.3)
AP (U/l)	4832 (4326–4981)	4352 (3919–4521)	1822 (1782–1863)	2598 (2316–2855)
Cortisol (µg/dL)	7.8 (1.1–19.9)	2.4 (1.0–12.0)	1.6 (1.1–2.1)	2.6 (1.9–3.2)
d-ROMs (UCARR)	27.6 (26.2–35.1)	33.4 (21.0-38.9)	93.6 (62.9-112.2)	99.9 (84.9-139.3)
BAP (µmol/L)	1120 (1043–1570)	1138 (502–2050)	920 (539–1330)	1622 (1419–1953)

TP: total proteins; AST: aspartate aminotransferase; ALP: alanine aminotransferase; GGT: gamma-glutamyl transferase; AP: alkaline phosphatase; d-ROMs: derivatives of reactive oxygen metabolites; BAP: biological antioxidant potential; FT0: within 8 h after birth; FT1: 10 days after birth. Values are reported as median and interquartile range (IQR)

to that of mares [36]. A protective role of the epitheliochorial placenta against ROS for the fetus during pregnancy and delivery has been proposed by Sgorbini et al. [37, 38]. Low levels of d-ROMs were found in our foals, while a higher amount was observed in maternal blood at parturition, confirming that hypothesis. Moreover, these levels are lower than those reported in jenny and mare foals, likely due to differences in breed, supplementation,

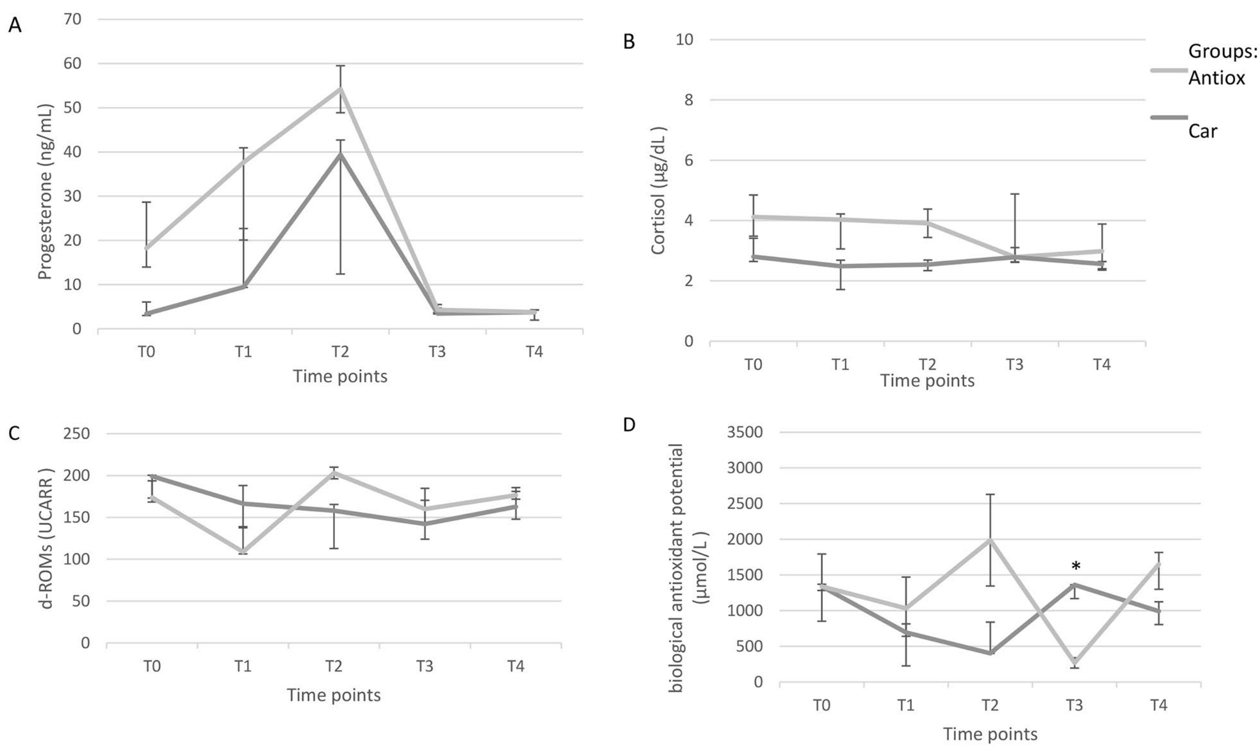

Fig. 1 Progesterone, cortisol, d-ROMs and BAP blood concentrations of mares of Car and Antiox groups. Line graphs show median and interquartile range (IQR: Q1, Q3). *indicates significant difference at $P \leq 0.05$ between group within each time point. T0: before the start of diet supplementation; T1: 15 days of diet supplementation; T2: at the end of diet supplementation; T3: within 8 h of foaling; T4: 10 days post-partum

or timing of blood collection [18, 37, 38]. The lack of variation in blood parameters and foal body weight at birth between the groups in this study may suggest that the placenta restricts the transfer of both oxidant and antioxidant molecules, along with their precursors and derivatives, to the fetus.

The concentration of BAP found in this study is in line with literature and confirms the low efficiency of the antioxidant system in foals at birth [38, 39]. It seems that the dietary supplementation of maternal antioxidants may not have been effective in improving that system. Although the results of the effect of antioxidants on blood variables in foals are controversial, direct comparisons among studies are difficult for differences in timing, amount and type of antioxidants and oxidative biomarkers used [16, 17, 29, 39]. In this study, we chose to utilize the BAP, which is one of the most extensively studied potential biomarkers of oxidant/antioxidant status in mares [18, 37, 40, 41]. Other studies have chosen to assess the serum concentration of antioxidants supplemented through diet [17, 19, 42].

Our results, however, showed a higher weight gain of foals born from mares that received mix of antioxidants, which is consistent with results observed in neonates and piglets [13, 29]. Nutrition of the mare during the last trimester of pregnancy plays an important role in the early development of the foal until three months of life [9, 10, 43]. Optimal concentrations of immunoglobulins and nutrients in the colostrum are paramount for neonate development [15]. Since the placenta acts as a barrier, the improved quality of colostrum found in mares after Oxyliver® supplementation could explain the better growth and development of neonates. Although the Brix quality was adequate in both groups, mare supplemental feeding with a mix of antioxidants increased the levels of immunoglobulins. Foals born with practically no immunity level and colostrum plays a crucial role in providing passive immunity and supporting the proper development of the foal's immune system [44].

Only few studies demonstrated that the maternal diet supplementation with antioxidants in the late pregnant mares resulted in an improvement of colostral immunoglobulins [16, 42]. Indeed, selenium and RRR-α -tocopherol administration in pregnant mares increased the concentration of IgG and IgM in the colostrum [42]. Vervuert [45] indicates as 80% of colostrum protein fraction is correlated to immunoglobulins G and A content. In our study, the colostral higher protein content along with a better Brix percentage, in the Antiox group compared to the Car group, indicate an increase in immunoglobulins. Furthermore, colostrum contains immune cells as leucocytes, bioactive substances such as lysozyme or lactoferrin, growth factors and hormones. All these components modulate metabolic processes such as the maturation of the gastrointestinal tract and meconium excretion in foals. Besides the role to provide immunoglobulins and bioactive substances, colostrum is the first and the most important feed of the new-born foal. Thus, further research into how to manipulate its composition through diet supplementation is clearly warranted. The higher weight gain of foals from mares that received Oxyliver® supplementation can be explained by the colostrum, which contain more bioavailable sources of energy and proteins, shown to affect growth rate [23]. Moreover, it was reported that reduced levels of fat and carbohydrates, such as lactose, have positive effects on long-bone mineral content preventing skeletal development problems [46, 47].

At the delivery (T3), the Antiox group showed lower levels of total proteins, albumin, glucose, creatinine, and urea in the blood compared to the Car group. Creatinine, total bilirubin, ALP, and AST were within the ranges reported for non-pregnant mares, but lower than the transient ranges reported in Standardbred mares at parturition (T3) [48]. Meanwhile, total proteins and albumin were lower than both the ranges reported for non-pregnant and parturient Standardbred mares [48]. These findings confirm that the synergistic effect of this combination of antioxidants enhances liver function, which could be beneficial for late pregnant mares experiencing a physiological hepatic loading [48, 49]. In this study, the synergistic effect of this combination of antioxidants favors the decrease in levels of proteins, albumin, and glucose in the blood, while increasing levels of proteins, immunoglobulins, casein, glucose, and free fatty acids in colostrum. The transfer of some elements from the mare plasma to the colostrum has been previously reported to depend on the concentration of antioxidants (vitamins E, A and β-carotene) in the plasma [50].

This mechanism could help explain the more paradoxical result of this study, the lower levels of antioxidants in Antiox group at delivery. The effect of Oxyliver® on liver efficiency may also promote the passage of antioxidants into the colostrum. Administration of antioxidants, that are contained in Oxyliver®, to late-pregnant mares resulted in an increased concentration of these molecules in colostrum and milk [29, 39, 42]. It would be interesting to examine the levels of antioxidants in colostrum to confirm this hypothesis.

Conclusions

In conclusion, these preliminary findings suggest that the diet supplementation with Oxyliver® from 290 to 320 days of gestation in Italian Salernitano mares is associated with a reduced gestation length and with an improvement in liver functions, ultimately enhancing colostrum quality. Since this study involved a limited group and the evaluated parameters are subject to various variables, it will be necessary to confirm whether those differences can be attributed to the supplement. Exploring the effects of Oxyliver® supplementation with various experimental designs (earlier and longer supplementation periods) on a larger population of mares, and potentially including those with compromised pregnancies, would be valuable avenues for further investigation.

Methods

Animals

From February to June 2023, eight late-term pregnant Italian Salernitano mares aged 12±3.1 years and owned by the CRII were involved in this study. Mares were stabled individually in approximately 9 m^2 boxes equipped with hay rack and individual feeder at night and spent the rest of the day (approximately 6–8 h/day) in an outdoor paddock (approximately 300–400 m^2). Mares and foals were turned out in small paddocks (approximately 100 m^2) for 2–4 h/day, starting from 72 h after foaling.

Experimental design

The following experimental design (double-blind, randomized, controlled trial) was established to assess whether supplementing the diet with antioxidants during the last 30 days of gestation affects mares and their foals' health and oxidative stress. The eight mares were randomly divided into two groups: a treated group (Antiox) that received a 30 g daily supplementation of a commercially available product Oxyliver® (Candioli Pharma, Turin, Italy) and a Car group that received the same amount of dehydrated carrot powder (Candioli Pharma, Turin, Italy), from day 290 to day 320 of gestation. Gestation days were calculated from the day of the last insemination/natural breeding of the mare. Antiox supplement is mix of antioxidants which contains: Vitamin C 150,000 mg; Vitamin E 75,000 IU; Choline chloride 3,500 mg; technically pure DL-Methionine 5,000 mg; Bitter orange extract 50,000 mg; Curcuma longa L 5,330 mg - Carduus marianus L. milk thistle extract 16,000 mg; Glycine 37,500 mg.; Colloidal Silica 85,000 mg; Lecithin 34,670 mg; Microcrystalline cellulose 10,670 mg.

Immediately before the start of diet supplementation (T0), after 15 (T1) and 30 days (T2) of dietary treatment, within 8 h of foaling (T3), and 10 days *post-partum* (T4) mares were evaluated for weight and BCS; a sample of blood was collected for subsequent analysis of haemato-biochemical parameters, progesterone and cortisol levels and oxidative/antioxidant status. In foals, weight measurements and blood collection for the evaluation of haemato-biochemical parameters, cortisol levels, and oxidant/antioxidant status were taken within 8 h after birth (TF0) and 10 days after (TF1). Moreover, the first milking colostrum was collected in all mares for determining quality and chemical composition.

At each time point before parturition, all mares underwent clinical examination, palpation and transrectal ultrasound examination to monitor the progress of the pregnancy. *Pre-partum* gynecological examination and transrectal ultrasound were performed with the mare restrained in stocks. The ultrasound was used to exclude the presence of abnormalities or pathologies by measuring the overall uterus-placental thickness (CTUP) and the echogenicity of fetal fluids or by estimating the heart rate as an index of fetal viability. In addition, gestation length (days), any parturition abnormalities, placental retention and neonatal disease were recorded.

Diets

A standard diet composed by *ad libitum* mixed hay (NDF: 68.81% DM; CP: 6.35% DM) and a commercial pelleted concentrate (Table 6) was administered to satisfy the nutritional requirement of the animals [22]. The concentrate was administered twice daily in function of the body weight of the mares, in the morning, before the mares were taken to the outdoor paddocks, and in the evening, after they returned to the box. Feed refusals were daily weighed. Each supplement was hand-mixed into the morning concentrate feeding; water was given *ad libitum*.

The Oxyliver® is composed by Maltodextrin (46%), Fructo-oligosaccharides (5%), Soya (bean) protein concentrate (1%), Palm oil, Products and co-products from processing fresh fruits and vegetables (Melon). Both feeds were administered at the rate of 30 g/d 30 days by personnel blinded to the composition of the feeds. The daily nutritional composition of the two dietary groups is provided in Table 6.

Table 6 Concentrate ingredients and nutritional characteristics of the diet administered to the two groups (Car and oxy)

Ingredients: wheat bran, wheat flour, corn flakes, oats, barley, barley flakes, carob, soybean meal, cane molasses, soybean oil, calcium carbonate, fava beans flakes, sodium chloride.

Group	Car		Antiox
DE	(MJ/kg DM)		6.68
CP	% DM		8.18
CF	% DM		45.79
NFE	% DM		39.86
AEE	% DM		0.8
Micronutrient intake*			
Vit C	mg/d	18.08	22.71
Vit E	IU/d	487.58	2739.6
Vit A	IU/d	28000.5	27310.5
Folic acid	mg	4.02	4.05

DE: digestible energy; DM: dry matter; CP: crude protein; CF: crude fiber; NFE: non-fiber extract; AEE: acid ether extract. * Micronutrient intake refers to the sum of pelleted concentrate and supplements (30 g/d of Car or Oxy)

BCS and weight

Mare weight and BCS measurements were collected at each time point, and the amount of grain mix offered was adjusted accordingly. Body weight was determined by electronic floor scales, and BCS was determined by 2 individuals (1 constant and 1 rotating) on a scale of 1 to 5 as described by Martin-Rosset & Younge [22] (1 = very thin; 5 = obese).

The average percentage change in the mare's body weight before and after parturition was calculated using the following formulas: WC1 (T2-T3) (%) = [(weight at T3 – weight at T2)/ weight at T2)*100]; WC2 (T3-T4)= [(weight at T4 –weight at T3)/ weight at T3)*100]. The percentage of weight gain of the foals was calculated in proportion of their weights at birth using the following formula: FWI (%) = [(weight at FT1- weight at FT0)/ weight at FT0) *100].

Colostrum samples and analysis

After parturition and before nursing, approximately 20 mL of colostrum were collected into a conical vial and stored at -20 °C until analysis. After thawing, the quality of each sample was immediately evaluated by Refractometer using Brix scale from 0 to 32% (Kerbl, Buchbach, DE). Chemical composition analysis (fat, protein, lactose, total solids, solids not fat, casein, urea, density, free fatty acids, glucose) was performed by a Fourier-transform infrared (FT-IR) spectrometer (MilkoScan™ FT3, FOSS, Italy).

Maternal and foals blood collection and analysis

Blood sampling was performed in each subject (mares and foals) at the mentioned time points using a 21-gauge needle and a 10 ml syringe from the jugular vein. Blood was collected in ethylenediaminetetraacetic acid (EDTA) and plain tubes (BD Vacutainer®, Becton Dickenson, USA). The tubes were transported refrigerated within 2 h to the certified (ISO 9001:2015) laboratory of the Veterinary Teaching Hospital of the University of Naples Federico II. The hematological examination was performed immediately with IDEXX ProCyte Dx™ analyzer (IDEXX Laboratories, Westbrook, ME, USA) to evaluate erythrocytes (M/μL), hematocrit (%), hemoglobin (g/dL), leucocytes (K/ μL), neutrophils (K/ μL), lymphocytes (K/ μL), monocytes (K/ μL), eosinophils (K/ μL), basophils (K/ μL), and platelets (K/ μL). To extract the serum the tubes were centrifugated at 3000 RPM for 10 min. The serum was stored at -20 °C until analysis for biochemical evaluation, cortisol, progesterone, and oxidative stress biomarkers, which was performed within 2 months. Biochemical evaluation was performed with the SAT450 clinical chemistry analyzer (KPM Analytics, Westborough MA, USA) to evaluate urea (mg/dL), creatinine (mg/dL), glucose (mg/dL), total proteins (PT; g/

dL), albumin (g/dL), total bilirubin (mg/dL), aspartate aminotransferase (AST; mg/dL), alanine aminotransferase (ALP; mg/dL), gamma-glutamyl transferase (GGT; mg/dL), alkaline phosphatase (AP; U/I). Blood cortisol (μg/dL) and progesterone (ng/mL) levels were assayed using an automated fluorescence enzyme immunoassay analyzer, the AIA®-360 system (TOSOH Bioscience, inc., South San Francisco, CA, USA). Oxidant/antioxidant status was determined by assessing derivatives of reactive oxygen metabolites (d-ROMs) and biological antioxidant potential (BAP). Measurements were performed using commercial Diacron® kits (Grosseto, Italy) according to the manufacturer's instructions and analyzed with the SAT450 (KPM Analytics, Westborough MA, USA). The d-ROMs results are expressed in Carratelli Units (UCARR) and 1 UCARR corresponds to 0.8 mg/L hydrogen peroxide (H_2O_2); whereas BAP is expressed in μmol/L of reduced iron.

Statistical analysis

All results were collected in a Microsoft Excel file and imported into a data analysis software Statistical Package for the Social Sciences- SPSS IBM® Statistics version 29.0 (IBM Corporation, Armonk, NY, USA). The distribution of the data was tested by Shapiro-Wilk normality test, which showed a non-normal distribution of many of the parameters considered. Therefore, given the non-normality of the data and the low sample size (4 Car vs. 4 Antiox), non-parametric tests were used. In particular, the Chi-square test was used to evaluate the differences in the sex of the foals born from the two groups, Mann-Whitney U test was performed to compare all parameters (e.g. weight, haemato-biochemicals values) between the two groups (Car vs. Antiox) at each evaluation time. Results are reported as median and interquartile range (IQR; Q1-Q3). Moreover, the effect of time on continuous parameters (haemato-biochemical parameters, progesterone, cortisol, d-ROMs and BAP concentrations) in each group (Car and Antiox) was evaluated by Friedman test, *post hoc* analysis with Wilcoxon's signed-rank test was used to compare time points. The differences in colostrum chemical composition were evaluated by one-way ANOVA considering the group as the fixed factor, and the HSD Tukey test. The significance level was set at $P<0.01$ and $P<0.05$.

Abbreviations

AEE	acid ether extract
ALP	Alanine aminotransferase
AP	alkaline phosphatase
AST	Aspartate aminotransferase
BAP	biological antioxidant potential
CF	crude fiber
CP	crude protein
CRII	Regional Institute for the Improvement of Equine Breeds in the Campania region (Centro Regionale di Incremento Ippico di Santa Maria Capua Vetere)
CTUP	combined uterus-placental thickness
DE	digestible energy
DM	dry matter
d-ROMs	Derivatives of reactive oxygen metabolites
EDTA	ethylenediaminetetraacetic acid
GGT	Gamma-glutamyl transferase
H_2O_2	Hydrogen peroxide
IQR	Interquartile range
NFE	non-fiber extract
ROS	Reactive oxygen species
SPSS	Statistical package for the social sciences
TP	Total proteins
UCARR	Carratelli Units

Acknowledgements

The authors would like to thank Candioli Pharma (Turin, Italy) for the technical support in formulating and for providing the used supplements. The authors would like to thank all the staff and students involved in the research activity, and in particular Francesca Valerio for her help in data collection and for the great dedication in this study.

Author contributions

Conceptualization and methodology, CDP, AV, MPP, MIC; formal analysis, AV; investigation, CM, NC; resources, MIC; data curation and writing—original draft preparation, CDP and AV; project administration, CDP; supervision and writing—review and editing, MPP, NC and MIC. All authors have read and agreed to the published version of the manuscript.

Funding

This research received no specific grant.

Data availability

Data is provided within the manuscript.

Declarations

Ethics approval and consent to participate

All procedures were carried out as part of routine clinical evaluations in accordance with the Guiding Principles in the Care and Use of Animals approved by Italian law, and the Directive 2010/63/EU. The study was approved by the Ethics Committee of the Animal Welfare Board OPBA (Centro Servizi Veterinari) of the University of Naples Federico II with protocol no. PG/2023/0011531 of 27/01/2023. Animal' owners gave informed consent to participate in the research.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Received: 4 April 2024 / Accepted: 26 August 2024
Published online: 11 September 2024

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