Italian Journal of Animal Science

ISSN: (Print) (Online) Journal homepage: www.tandfonline.com/journals/tjas20

Dietary mineral and vitamin supplementation improved the reproduction, the haematology, and some circulating biochemical's of cyclic East-Bulgarian and Arabian mares

Maya Popova, Radka Malinova, Vasil Nikolov, Boyko Georgiev, Paulina Taushanova & Maria Ivanova

To cite this article: Maya Popova, Radka Malinova, Vasil Nikolov, Boyko Georgiev, Paulina Taushanova & Maria Ivanova (2021) Dietary mineral and vitamin supplementation improved the reproduction, the haematology, and some circulating biochemical's of cyclic East-Bulgarian and Arabian mares, Italian Journal of Animal Science, 20:1, 728-735, DOI: 10.1080/1828051X.2021.1910581

To link to this article: https://doi.org/10.1080/1828051X.2021.1910581

Published online: 21 Apr 2021.

ITALIAN JOURNAL OF ANIMAL SCIENCE
2021, VOL. 20, NO. 1, 728–735
https://doi.org/10.1080/1828051X.2021.1910581

PAPER

Dietary mineral and vitamin supplementation improved the reproduction, the haematology, and some circulating biochemical's of cyclic East-Bulgarian and Arabian mares

Maya Popova[a], Radka Malinova[a], Vasil Nikolov[a], Boyko Georgiev[b], Paulina Taushanova[b] and Maria Ivanova[b]

[a]Agricultural University–Plovdiv, Plovdiv, Bulgaria; [b]Institute of Biology and Immunology of Reproduction, Sofia, Bulgaria

ABSTRACT

This study aimed to investigate the dietary mineral and vitamin supplementation on the hematological, the circulating biochemical parameters, and the reproductive performance of cyclic mares aged 4–12 years old. Purebred Arabian (PAB, $n = 8$) and East-Bulgarian (EBB, $n = 12$) breeds were equally divided into treated and control groups. The nutritional supplement was given orally (50 g daily/animal/day) for seven consecutive days and repeated for 4 consecutive months. All mares were examined daily with ultrasound from exhibiting oestrus signs till ovulation. Blood samples were collected with and without anticoagulant before supplementation, on Day 7 and Day 120 for the measurement of ALT, lactate dehydrogenase, bilirubin, calcium, phosphorus, and creatinine and to perform full blood picture. The nutritional supplement decreased lactate dehydrogenase ($p < .05$) and creatinine ($p < .05$). Compared to the PAB mares, control mares had one day shorter oestrous cycle length (20.2 ± 2.13 vs 21.0 ± 2.46 days), but the treated EBB mares had two days shorter oestrus cycle (20.00 ± 1.202 vs 22.00 ± 0.93 days). The oestrus phase of the treated mares was shorter ($p < .001$) than that of the control mares (3.63 ± 0.38 vs 5.50 ± 0.31 days). The diameter of the dominant follicle varied ($p < .05$) between the two breeds that was 38.25 ± 1.90 and 36.94 ± 1.70 mm for treated and control EBB but was 35.38 ± 1.41 and 34.89 ± 1.35 mm for treated and control PAB. In conclusion, the nutritional supplement had a good effect on the reproductive status, and on the blood parameters (hematological and biochemical) of the mares, but more research is necessary to recommend its use.

ARTICLE HISTORY
Received 16 October 2020
Revised 16 March 2021
Accepted 25 March 2021

KEYWORDS
Blood parameters; oestrous cycle; mares; nutritional supplementation; ovulation

HIGHLIGHTS

- The addition of the nutritional supplementation to the mares from the East-Bulgarian breed lead to shorter oestrus.
- There was a tendency to shorten the sexual cycle (up to 2 days) and increase the size of the dominant follicles in mares receiving the supplement.

Introduction

The East-Bulgarian horse breed (EBB) was established in Bulgaria in 1951. It currently has the status of endangered. The stud book of the breed was recognised by the World Breeding Federation for Sport Horses (WBFSH) in 2012 and was closed on the maternal line. In the last three generations, the selection aimed at changing the constitutional-productive type – from running to more massive, suitable for the classical disciplines of equestrian sports (Sabeva et al. 2018; Sabeva and Popova 2019). The Purebred Arabian breed (PAB) is widespread throughout the world and has played a major role in the creation of a number of breeds. Since 1998, the Bulgarian population of Purebred Arabian horses has been approved and accepted into the World Arabian Horse Organisation (WAHO) (Popova 2017). Hematological and biochemical parameters of the blood were used in horses for the clinical diagnosis of metabolic diseases, infectious, some parasitic diseases, etc.; monitoring the recovery during treatment, assessing metabolic conditions. Blood parameters can be influenced by a number of factors related to the physiological condition of horses like age, season, nutrition, reproductive status, geographical location, breed and more. In this connection, Cebulj-Kadunc et al. (2002), Gurgoze and Icen (2010)

CONTACT Mrs Maya Popova m_popova1@abv.bg Agricultural University–Plovdiv, Plovdiv, Bulgaria

had analysed the influence of the age on biochemical and hematological parameters in horses. Light riding horses have higher red blood cells (RBC), haemoglobin (HB) and blood cell volume (PCV) than heavy horse breeds (Jain1993; Kramer 2000; Grondin and Dewitt 2010). Yur et al. (2008) found that the addition of vitamin E and selenium to the ration had a significant effect on serum concentrations of calcium, phosphorus, iron and the copper/zinc ratio. These factors have a direct impact on the reproductive functions of mares. The diameter of the Graafian follicle is influenced by age (Morel et al. 2010), season (Gastal et al. 2007), and multiple ovulation (Ginther et al. 2009). Abo-El-Maaty et al. (2013) found that the diameter of the dominant follicle and the first subordinate follicle were larger in mares treated with mineral supplements than in control mares. The size of the dominant follicle, in turn, can serve as an indirect indicator of the quality of the released egg (Bruck 1996).

In an experiment with 18 mares from the Thoroughbred breed, Popov and others (1974) found that nutrition with granular feed mixture, enriched with micronutrients and vitamins during pregnancy and lactation had a beneficial effect on mares during pregnancy and on development of the foals. The aim of the present study was to establish the influence of non-hormonal food supplement on hematological and biochemical parameters, as well as on the reproductive status of the Purebred Arabian and the East-Bulgarian mares.

Materials and methods

Animals and experimental design

The experiment was conducted in the State Stud 'Kabiuk', near the town of Shumen, Bulgaria, and included Purebred Arabian (PAB, $N=8$) and the Eastbulgarian (EBB, N-12). breeds. Mares in each breed were divided into two equal groups (control and treated or supplemented) by the method of analogues (by age, body measurements, reproductive and health status). The experiment started from March to June during 2019. The average body weight of the East-Bulgarian mares ranges from 440 to 500 kg. The Arabian mares average body weight ranges from 340 to 380 kg.

The mares in both groups were fed a ration that contained rolled concentrated feed (a mixture of triticale, corn and black oats) and haylage. Haylage has a dry matter content of 33.23%, which includes crude protein of 32.17%, crude cellulose of 27.57%, crude fat of 4.58%, nitrogen-free extracts (NFE) of 28.3%, and crude ash of 7.38%. One kilogram of haylage contains Calcium (Ca) of 5.45 g, Magnesium (Mg) of 1.37 g, Copper (Cu) of 6.84 mg, iron (Fe) of 0.36 g, Zinc (Zn) of 23.19 mg, and Manganese (Mn) of 52.91 mg. The dry matter in the concentrated feed was 91.66%, as in it the crude protein was 12.27%, the crude cellulose was 11.16%, the crude fat was 4.66%, nitrogen-free extracts was 69.65%, and crude ash was 2.26%. In one kilogram of concentrated feed, the content of Ca was 61.85 mg, of Mg was <1.00 mg, Cu was 2.89 mg, Fe was 2.24 mg, Zn was 1.40 mg, and Mn was 0.20 mg. At the morning ration, mares from the experimental groups received an additional nutritional supplement, 50 grams per mare daily for one week per month, for the duration of 4 months. The supplement contained: crude fibre (0.1%), crude protein (0.6%), crude fat (0.1%), crude ash (18.3%), Zn (sulfate, monohydrate, 2500 mg); Mn (sulfate, monohydrate, 1500 mg); Fe (sulfate, monohydrate, 1500 mg); Cu (sulfate, monohydrate, 200 mg), Selenium (Se, 10 mg), Vit.A (1,000,000 U), Vit.D3 (50,000 U), Vit. E (5000 mg), Nacinamide (3000 mg), Calciu-D-pantpthenate (750 mg), Vit.B2(460 mg), Vit.K3 (285 mg), Vit.B6 (250 mg), Vit.B9 (50 mg), Vit.B12 (25.000 μg), Biotin (11.000 μg), L-lysine (HCL, 450 mg), DL methionine (1.000 mg), Calcium propionate (139.000 mg), Citric acid (12.500 mg). Animals had constant access to water.

Blood sampling and ultrasound examination

Three blood samples were collected in vaccum tubes with and without anticoagulant from all mares via Jugular vein puncture between 6 and 8 h in the morning before meals before the start of supplementation (Day 0), Day7 after supplementation, and at the end of supplementation (Day 120).

A portable ultrasound machine 'WED3000V' (China) equipped with a linear transducer with a frequency of 6.5 MHz was used to monitor the effect of the dietary supplement on the reproductive capacity of mares, the reproductive status (duration of sexual cycle; duration of oestrus; ovulation; size of the follicle) was monitored monthly during the duration of the experiment.

Whole blood and biochemical analysis

The tested the blood parameters and the Haematological parameters WBC, Lym, Mon, Gran, RBC, MCV, Hct, MCH, MCHC, RDW, Hb, THR, MPV, PDW, were examined using MS4 + apparatus (Switzerland) with veterinary software for horses. The biochemical analyzes were performed

Table 1. Mean values of hematological and biochemical parameters of 10 experimental mares ($n = 4$ Purebred Arabian breed (PAB) + 6 East-Bulgarian horse breed (EBB)) and the 10 ($n = 4$ PAB+ 6 EBB) control mares.

	Day 0		Day 7		Day 120	
Parameters	Treated	Control	Treated	Control	Treated	Control
RBCs, m/mm^3	8.06 ± 0.19	8.03 ± 0.20	7.72 ± 0.21	7.79 ± 0.22	8.06 ± 0.48	7.24 ± 0.53
Hb, g/L	11.76 ± 0.68	10.93 ± 0.72	9.61 ± 0.32	9.73 ± 0.33	10.44 ± 0.57	9.02 ± 0.63
THR, m/mm^3	210.9 ± 49.89	324.0 ± 52.59	458.9 ± 132.31	629.5 ± 139.5	273.1 ± 45.32	410.9 ± 50.67
WBCs, 1000/μL	7.75 ± 0.84	10.04 ± 0.89	6.96 ± 1.43	10.34 ± 1.50	7.05 ± 0.68	6.99 ± 0.76
Lym %	37.48 ± 3.00	28.46 ± 3.16	43.99 ± 2.74	42.33 ± 2.89	42.92 ± 5.81	50.86 ± 6.50
Gran %	57.88 ± 5.80	59.40 ± 6.12	51.16 ± 2.95	52.99 ± 3.11	53.60 ± 5.94	43.79 ± 6.64
Mono %	4.52 ± 0.39	3.25 ± 0.41	4.89 ± 0.67	4.62 ± 0.71	3.17 ± 0.33	3.70 ± 0.37
MCV, fl	44.38 ± 1.31	44.64 ± 1.39	46.59 ± 0.76	47.29 ± 0.80	48.27 ± 1.06	44.92 ± 1.19
Hct %	35.70 ± 2.28	33.71 ± 2.40	35.97 ± 1.24	36.75 ± 1.31	39.20 ± 2.00	34.65 ± 2.24
MCH, pg	12.02 ± 0.19	12.63 ± 0.20	12.33 ± 0.19	12.38 ± 0.21	12.62 ± 0.35	11.66 ± 0.39
MCHC, g/dL	27.28 ± 0.32	27.51 ± 0.33	26.66 ± 0.29	26.44 ± 0.31	26.12 ± 0.71	26.09 ± 0.80
MPV, fl	7.81 ± 0.27	7.83 ± 0.28	7.68 ± 0.12	7.89 ± 0.13	7.21 ± 0.18	7.48 ± 0.20
RDW %	19.25 ± 1.41	15.61 ± 1.49	14.24 ± 0.27	14.21 ± 0.29	13.40 ± 0.43	13.41 ± 0.49
PDW %	8.77 ± 0.46	9.60 ± 0.48	9.78 ± 0.34	10.41 ± 0.36	8.83 ± 0.49	9.66 ± 0.55
ALT U/L	56.73 ± 16.44	90.63 ± 17.32	42.88 ± 8.59	38.00 ± 9.05	59.00 ± 8.23	40.00 ± 9.21
LDH U/L	1302 ± 117.3	1598 ± 123.65	1183 ± 105.9	1233 ± 111.6	1117 ± 89.62	1292 ± 100.2
Creatinine, μmol/L	248.2 ± 61.66	352.9 ± 65.00	131.1 ± 6.88	134.6 ± 7.25	116.1 ± 6.08	124.3 ± 6.80
Bilirubin, μmol/L	82.30 ± 15.85	63.69 ± 16.71	38.76 ± 9.78	29.99 ± 10.31	37.01 ± 8.44	28.75 ± 9.44
Calcium, mmol/L	1.88 ± 0.65	2.48 ± 0.69	0.92 ± 0.16	0.83 ± 0.17	0.79 ± 0.18	1.18 ± 0.20
Phosphorus, mmol/L	5.94 ± 0.81	6.75 ± 0.85	2.66 ± 0.37	2.68 ± 0.39	3.08 ± 0.42	3.30 ± 0.47

RBCs: Red blood cells; Hb: haemoglobin; THR: platelets; WBCs: white blood cells; Lym: lymphocytes; Gran: granulocytes; Mono: monocytes; MCV: mean corpuscular volume; Hct: haematocrit; MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobin concentration; MPV: mean platelet volume; RDW: red cell distribution width; PDW: platelet distribution width; ALT: alanine aminotransferase; LDH: lactate dehydrogenase.

spectrophotometrically on an Mindray BA88 A apparatus, using the following parameters and reagents: ALT-Tris buffer, 100 mM; pH 7.5, L Alanine 500 mM, 2Oxoglutarate 15 mM, NADH 0.18 mM, LDH ≥1700 U/l and wavelength 340 nm; Ca-CPC 0.14 mM, 8-quinolinol 25 mM, HCl pH 1.20 and wavelength 575 nm; Creatinine-picnic acid 0.14 mM, NaOH 0.18 M, Na tetraborate 10 mM and wavelength 510 nm; LDH-phosphate buffer 50 mM, pH 7.5, sodium pyrivate 0.60 mM, NADH 0.18 mM, wavelength 340 nm; P-ammonium molybdate 0.4 mmol/l, sulphuric acid 0.21 mol/l, wavelength 340 nm; Total bilirubin-hydrochloric acid 0.1 M, 3,5-dichlorophenyldiazonium salt 2 mM, wavelength 510 nm.

Statistical analyses

Single-factor and multi-factor analysis of variance performed with the SPSS 21 software product were used for data processing.

The following linear model was used to account for the effect of the food supplement on the hematological parameters:

$$Yijk = \mu + SZi + SXj + AGk + Bkj + eijk \quad \text{(M1)}$$

where *Yijk* – observation vector; μ – total average constant; *SZi*, *SXj* and *AGk* were fixed effects of the breed, respectively ($i = 2$); the blood sample ($j = 3$: 1 – initial stage; 2 – intermediate stage; 3 – final stage) and the group ($k = 2$: 1 – experimental; 2 – control); *Bkj* was the random effect of the blood sample within the group; *eijk* – residual variant.

To account for the effect of the food supplement on the duration of oestrus, the sexual cycle and the size of the Graafian follicle:

$$Yik = \mu + Gi + eik, \quad (2)$$

$$Yijk = \mu + Gi + Bj + GBij + eijk, \quad (3)$$

where *Y* (…) – vector of observation; μ – total average constant; *Gi* and *Bj*, – were fixed effects of the *i*-th group and the *j*-th breed, respectively; *GB* was the random effect of the *i*-th group within the *j*-th breed; *e* (…) – residual variance.

Results

In Table 1, the average values of the hematological and biochemical parameters of the blood from the control and the experimental groups of the mares of both breeds, the object of the research, are presented. The table shows that the levels of alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) were higher than the reference values indicated by Mircheva (2006) – 3–25 U/L and 130–470 U/L, respectively. In mares in the experimental group, LDH levels decreased compared to those in the control group from the beginning to the end of the experiment, while ALT levels decreased at the end of the 1st week and increased at the end of the experiment.

In the mares that were studied, creatinine (Creat) decreased 50% in the first week. At the end of the

Table 2. Influence of the nutritional supplement on the haematological and biochemical parameters of the blood in mares from Purebred Arabian breed (PAB) and East-Bulgarian horse breed (EBB) (F-criterion and degree of reliability).

	Day 0		Day 7		Day 120	
Parameters	Group	Breed in the group	Group	Breed in the group	Group	Breed in the group
df	1	2	1	2	1	2
RBCs, m/mm^3	0.020 (0.889)	3.058 (0.049)	0.049 (0.828)	1.338 (0.296)	1.322 (0.277)	0.450 (0.650)
Hb, g/L	0.702 (0.417)	6.501 (0.011)	0.059 (0.811)	0.943 (0.414)	2.787 (0.126)	0.364 (0.704)
THR, m/mm^3	2.431 (0.143)	1.102 (0.361)	0.787 (0.391)	1.668 (0.227)	4.110 (0.040)	0.411 (0.674)
WBCs, 1000/μL	3.498 (0.048)	2.341 (0.135)	2.643 (0.128)	0.697 (0.516)	0.004 (0.953)	0.449 (0.651)
Lym %	4.277 (0.042)	0.320 (0.732)	0.174 (0.683)	1.160 (0.344)	0.829 (0.384)	1.012 (0.398)
Gran %	0.033 (0.859)	0.474 (0.633)	0.182 (0.677)	0.616 (0.555)	1.213 (0.296)	0.671 (0.533)
Mono %	5.066 (0.034)	0.904 (0.429)	0.082 (0.780)	1.005 (0.393)	1.159 (0.307)	0.126 (0.883)
MCV, fl	0.018 (0.896)	0.021 (0.979)	0.393 (0.542)	1.343 (0.295)	4.419 (0.044)	0.074 (0.929)
Hct %	0.359 (0.560)	0.849 (0.450)	0.189 (0.671)	0.558 (0.586)	2.293 (0.161)	0.078 (0.925)
MCH, pg	4.814 (0.045)	0.680 (0.524)	0.028 (0.869)	1.352 (0.293)	3.483 (0.050)	0.673 (0.532)
MCHC, g/dL	0.259 (0.619)	4.937 (0.039)	0.267 (0.614)	0.374 (0.695)	0.001 (0.975)	0.076 (0.928)
MPV, fl	0.001 (0.978)	0.211 (0.812)	1.423 (0.254)	0.250 (0.783)	0.934 (0.357)	0.078 (0.926)
RDW %	3.157 (0.050)	6.504 (0.011)	0.005 (0.943)	0.032 (0.969)	0.000 (0.994)	0.959 (0.416)
PDW %	1.540 (0.237)	2.881 (0.050)	1.628 (0.224)	0.225 (0.802)	1.254 (0.289)	1.403 (0.290)
ALT U/L	2.015 (0.179)	2.155 (0.155)	0.153 (0.702)	0.972 (0.404)	2.366 (0.155)	0.529 (0.605)
LDH U/L	3.012 (0.106)	8.941 (0.004)	0.106 (0.749)	2.746 (0.101)	1.684 (0.224)	4.421 (0.042)
Creatinine, μmol/L	1.364 (0.264)	2.461 (0.124)	0.125 (0.729)	2.766 (0.100)	0.813 (0.389)	3.037 (0.045)
Bilirubin, μmol/L	0.652 (0.434)	1.438 (0.273)	0.381 (0.548)	0.107 (0.899)	0.426 (0.529)	0.140 (0.871)
Calcium, mmol/L	0.393 (0.542)	2.061 (0.167)	0.132 (0.722)	2.204 (0.150)	2.008 (0.187)	2.460 (0.135)
Phosphorus, mmol/L	0.475 (0.503)	0.063 (0.939)	0.003 (0.961)	1.066 (0.373)	0.119 (0.737)	2.475 (0.134)

RBCs: Red blood cells; Hb: haemoglobin; THR: platelets; WBCs: white blood cells; Lym: lymphocytes; Gran: granulocytes; Mono: monocytes; MCV: mean corpuscular volume; Hct: haematocrit; MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobin concentration; MPV: mean platelet volume; RDW: red cell distribution width; PDW: platelet distribution width; ALT: alanine aminotransferase; LDH: lactate dehydrogenase.

experiment, it was lower in the treated mares and in both groups it was within the accepted norms.

In the mares of this study, bilirubin returned to normal values after 1 week, and in the control group it was lower than in the experimental group until the end of the study.

Calcium (Ca) in the blood decreased and was below normal at the end of the experiment in both groups. In both groups, phosphorus (P) levels exceeded the upper limit of the reference values reported in the literature (Mircheva 2006). At the beginning of the experiment in both groups it was higher, but tended to decrease, and in treated mares at the end of the experiment it was lower than in untreated mares.

At the beginning of the experiment, lymphocytes (Lym) were normal in both groups, with higher values in the experimental group. After the 1st week and at the end of the experiment, the number of Lym in the experimental group mares had lower values close to the norm, while in the control group they almost doubled.

At the beginning of the experiment, the width of the erythrocyte distribution (RDW) was also slightly increased in both groups, but at the end of the experiment they returned to normal, as in the mares of the experimental group they were slightly lower than in the control group. Platelets (THR) were normal in both groups, but doubled after the 1st week of the experiment. At the end of the study, the values in the experimental group returned to normal, while in the control group they remained high.

The nutritional supplement had a significant effect on the haematological and biochemical parameters of the blood (Table 2), and the effect was diverse. At the beginning of the experiment, the experimental and control groups differed significantly in some of the studied indicators. WBC ($p < .05$) and MCH ($p < .05$) had higher values in the control group, and Lym ($p < .05$), Mon ($p < .05$) and RDW ($p < .05$) – in the experimental group.

During the intermediate stage of the experiment, the differences were eliminated, and at the end of the experiment significant differences were again observed – in MCH ($p < .05$), MCV ($p < .05$) and THR ($p < .05$), as the first two indicators were higher in the experimental group and the latter ones in the control group.

The same patterns were observed with respect to the breeds, within the groups. At the beginning of the experiment, the breeds differed significantly in the level of LDH ($p < .01$), PDW ($p < .05$), MCHC ($p < .05$), RBC ($p < .05$), RDW ($p < .05$), Hb ($p < .05$), as in both breeds the values of the first 3 indicators were higher in the control group, and the rest in the experimental group (Tables 3 and 4).

The addition of nutritional supplement to the ration of mares, according to the attached scheme, had a significant effect on the duration of oestrus, but only

Table 3. Mean values of hematological and biochemical parameters of Purebred Arabian breed (PAB) mares from the experimental ($n = 4$) and control groups ($n = 4$).

	Day 0		Day 7		Day 120	
Parameters	Treated	Control	Treated	Control	Treated	Control
RBCs, m/mm^3	8.53 ± 0.29	8.14 ± 0.29	7.93 ± 0.31	8.07 ± 0.31	8.51 ± 0.76	7.27 ± 0.76
Hb, g/L	14.15 ± 1.02	10.33 ± 1.02	10.03 ± 0.47	9.88 ± 0.47	10.92 ± 0.90	9.10 ± 0.90
THR, m/mm^3	241.8 ± 74.37	395.0 ± 74.37	359.8 ± 197.2	861.8 ± 197.2	239.7 ± 71.66	437.8 ± 71.66
WBCs, 1000/μL	8.24 ± 1.25	8.19 ± 1.25	6.97 ± 2.13	12.11 ± 2.13	7.11 ± 1.08	7.71 ± 1.08
Lym %	38.80 ± 4.47	30.58 ± 4.47	44.78 ± 4.09	46.65 ± 4.09	40.51 ± 9.19	42.01 ± 9.19
Gran %	56.48 ± 8.65	65.18 ± 8.65	50.90 ± 4.40	49.55 ± 4.40	56.14 ± 9.39	50.94 ± 9.39
Mono %	4.83 ± 0.58	2.80 ± 0.58	4.33 ± 1.01	3.80 ± 1.01	3.00 ± 0.52	3.72 ± 0.52
MCV, fl	44.13 ± 1.96	44.73 ± 1.96	47.03 ± 1.14	46.05 ± 1.14	48.26 ± 1.68	45.38 ± 1.68
Hct %	37.48 ± 3.40	36.23 ± 3.40	37.25 ± 1.85	37.03 ± 1.85	39.89 ± 3.16	35.07 ± 3.16
MCH, pg	11.80 ± 0.28	12.65 ± 0.28	12.48 ± 0.29	12.08 ± 0.29	12.74 ± 0.55	11.23 ± 0.55
MCHC, g/dL	26.88 ± 0.47	28.48 ± 0.47	26.88 ± 0.44	26.58 ± 0.44	26.23 ± 1.13	25.80 ± 1.13
MPV, fl	7.99 ± 0.40	7.80 ± 0.40	7.65 ± 0.18	7.80 ± 0.18	7.26 ± 0.29	7.54 ± 0.29
RDW %	24.20 ± 2.10	14.40 ± 2.10	14.30 ± 0.41	14.25 ± 0.41	13.38 ± 0.69	14.08 ± 0.69
PDW %	8.93 ± 0.68	10.75 ± 0.68	9.93 ± 0.51	10.23 ± 0.51	9.57 ± 0.78	10.06 ± 0.78
ALT U/L	83.25 ± 24.50	68.00 ± 24.50	48.75 ± 12.81	27.00 ± 12.81	66.00 ± 13.02	34.67 ± 13.02
LDH U/L	971.3 ± 174.9	1208 ± 174.9	950.5 ± 157.8	1143 ± 157.8	1296 ± 141.7	1514 ± 141.7
Creatinine, μmol/L	303.4 ± 91.92	220.9 ± 91.92	140.7 ± 10.26	120.9 ± 10.26	106.9 ± 9.61	137.6 ± 9.61
Bilirubin, μmol/L	55.55 ± 23.63	66.55 ± 23.63	43.28 ± 14.58	29.65 ± 14.58	33.00 ± 13.35	30.97 ± 13.35
Calcium, mmol/L	1.32 ± 0.98	1.21 ± 0.9	0.62 ± 0.24	0.99 ± 0.24	0.91 ± 0.29	1.61 ± 0.29
Phosphorus, mmol/L	5.84 ± 1.21	6.47 ± 1.21	2.16 ± 0.56	2.44 ± 0.56	3.73 ± 0.66	4.04 ± 0.66

RBCs: Red blood cells; Hb: haemoglobin; THR: platelets; WBCs: white blood cells; Lym: lymphocytes; Gran: granulocytes; Mono: monocytes; MCV: mean corpuscular volume; Hct: haematocrit; MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobin concentration; MPV: mean platelet volume; RDW: red cell distribution width; PDW: platelet distribution width; ALT: alanine aminotransferase; LDH: lactate dehydrogenase.

Table 4. Mean values of hematological and biochemical parameters of East-Bulgarian horse breed (EBB) mares from the experimental ($n = 6$) and control groups ($n = 6$).

	Day 0		Day 7		Day 120	
Parameters	Treated	Control	Treated	Control	Treated	Control
RBCs, m/mm^3	7.60 ± 0.26	7.91 ± 0.29	7.52 ± 0.27	7.51 ± 0.31	7.61 ± 0.59	7.21 ± 0.76
Hb, g/L	9.36 ± 0.91	11.53 ± 1.02	9.20 ± 0.42	9.57 ± 0.47	9.96 ± 0.70	8.93 ± 0.90
THR, m/mm^3	180.2 ± 66.52	253.0 ± 74.37	558.2 ± 176.4	397.3 ± 197.2	306.4 ± 55.51	384.0 ± 71.66
WBCs,1000/μL	7.27 ± 1.12	11.89 ± 1.25	6.96 ± 1.90	8.56 ± 2.13	6.99 ± 0.84	6.27 ± 1.08
Lym %	36.16 ± 4.00	26.35 ± 4.47	43.20 ± 3.66	38.00 ± 4.09	45.33 ± 7.12	59.70 ± 9.19
Gran %	59.28 ± 7.74	53.63 ± 8.65	51.42 ± 3.93	56.43 ± 4.40	51.06 ± 7.28	36.63 ± 9.39
Mono %	4.22 ± 0.52	3.70 ± 0.58	5.46 ± 0.90	5.43 ± 1.01	3.33 ± 0.41	3.68 ± 0.52
MCV, fl	44.64 ± 1.75	44.55 ± 1.96	46.16 ± 1.02	48.53 ± 1.14	48.29 ± 1.30	44.47 ± 1.68
Hct %	33.92 ± 3.04	31.20 ± 3.40	34.68 ± 1.66	36.48 ± 1.85	38.50 ± 2.45	34.23 ± 3.16
MCH, pg	12.24 ± 0.25	12.60 ± 0.28	12.18 ± 0.26	12.68 ± 0.29	12.50 ± 0.42	12.08 ± 0.55
MCHC, g/dL	27.68 ± 0.42	26.55 ± 0.47	26.44 ± 0.39	26.30 ± 0.44	26.02 ± 0.87	26.37 ± 1.13
MPV, fl	7.64 ± 0.36	7.85 ± 0.40	7.70 ± 0.16	7.98 ± 0.18	7.17 ± 0.22	7.41 ± 0.29
RDW %	14.30 ± 1.88	16.83 ± 2.10	14.18 ± 0.36	14.18 ± 0.41	13.42 ± 0.53	12.73 ± 0.69
PDW %	8.62 ± 0.61	8.45 ± 0.68	9.64 ± 0.45	10.60 ± 0.51	8.09 ± 0.60	9.25 ± 0.78
ALT U/L	30.20 ± 21.91	113.25 ± 24.50	37.00 ± 11.45	49.00 ± 12.81	52.00 ± 10.08	45.33 ± 13.02
LDH U/L	1633 ± 156.4	1987 ± 174.9	1416 ± 141.1	1323 ± 157.8	939.8 ± 109.7	1070 ± 141.7
Creatinine, μmol/L	193.0 ± 82.22	484.8 ± 91.92	121.5 ± 9.17	148.4 ± 10.26	125.3 ± 7.45	111.1 ± 9.61
Bilirubin, μmol/L	109.0 ± 21.14	60.83 ± 23.63	34.24 ± 13.04	30.33 ± 14.58	41.02 ± 10.34	26.53 ± 13.35
Calcium, mmol/L	2.44 ± 0.87	3.75 ± 0.98	1.22 ± 0.21	0.68 ± 0.24	0.67 ± 0.22	0.75 ± 0.29
Phosphorus, mmol/L	6.04 ± 1.08	7.03 ± 1.21	3.15 ± 0.50	2.93 ± 0.56	2.43 ± 0.51	2.55 ± 0.66

RBCs: Red blood cells; Hb: haemoglobin; THR: platelets; WBCs: white blood cells; Lym: lymphocytes; Gran: granulocytes; Mono: monocytes; MCV: mean corpuscular volume; Hct: haematocrit; MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobin concentration; MPV: mean platelet volume; RDW: red cell distribution width; PDW: platelet distribution width; ALT: alanine aminotransferase; LDH: lactate dehydrogenase.

in mares from EBB (Table 5). This was established by both one-factor and multifactor analysis. In the experimental group of EBB oestrus was 1.87 days (66%) shorter than in the control group (Table 6). In PAB, the mares in the experimental group had a slightly prolonged oestrus (0.14 days), and the difference with the control group was insignificant.

The study also found significant breed differences ($p < .05$) in the size of the Graafian follicle (Table 5). In the experimental groups, the size of the follicle in EBB was 2.87 mm (8.1%) larger than in PAB. In the control groups, the difference was 2.05 mm (5.9%), again in favour of the EBB.

Discussion

According to Mircheva (2006), ALT is rarely an indicator of clinical reliability, unless it exceeds at least twice the upper limit of the reference values, as the results at the end of this experiment. The LDH values of the studied mares were close to those reported by Aros et al. (2017) – 807 ± 515 U/L (from 353 to 1746 U/L) and from Winnicka (2011) – 520–1480 U * L-1.

Alanine aminotransferase (ALT) is an enzyme that is released from the cytoplasm of hepatocytes in the plasma when they are destroyed and is considered very specific for the liver. The results obtained by us at the end of the experiment show that the mares from both groups have doubled ALT values, which could indicate stress and liver problems. Lactate dehydrogenase is an enzyme responsible for the anaerobic conversion of pyruvate to lactate. An important advantage of LDH analysis is that its levels remain elevated for several days after the onset of muscle damage (Mircheva 2006).

Creatinine is a product of the breakdown of creatinine phosphate in muscle tissue. It is excreted exclusively by glomerular filtration through the kidneys. If the glomerular filtration of the kidneys is impaired, the amount of creatinine in the serum increases (Mircheva 2006). The reasons why creatinine was elevated before the experiment could be muscle trauma, kidney damage or inflammation.

The results for Creatinine were close to those reported by other authors – Meyer and Harvey (2004), Boediker (1991)), with values ranging from 88.4 to 167.96 μmol/L.

Mircheva (2006) states that the total serum bilirubin in horses and ponies varies between 1 and 2 mg/dL (17–34 μM/L) and depends on the last food intake.

Table 5. Influence of the nutritional supplement on the reliability of the differences between the experimental and control group, and the breed within the group, the duration of oestrus, sexual cycle and the size of the Graafian follicle in mares (F-criterion and degree of reliability).

Sign		Oestrous	Sexual cycle	Size of the follicle
Model	Factor	F–criterion and degree of reliability		
		Purebred Arabian breed		
Model 2	Group	0.118 (0.734)	0.053 (0.827)	0.063 (0.805)
		Eastbulgarian breed		
Model 2	Group	14.55 (0.001)	1.731 (0.236)	0.264 (0.614)
		Multivariate linear model		
Model 3	Group	0.254 (0.630)	0.285 (0.610)	0.022 (0.886)
	Breed	3.243 (0.115)	0.011 (0.918)	4.591 (0.043)
	Group withinBreed	7.104 (0.032)	0.285 (0.610)	0.022 (0.886)

Calcium in treated mares it was lower than reported by other authors: Padilha et al. (2017) −13.22 ± 0.59 mgq/dL in the breed Brazilian sport horse; Aros et al (2017) −4.5 ± 3.4 mmol/L in native workhorses in Chile.

Infraction of the renal function is often associated with mild hypocalcaemia accompanied by an increase in inorganic phosphate concentrations. Hypocalcaemia is most often due to loss of calcium in the urine or insufficient movement of calcium from the bones to the blood. Other reasons may be insufficient consumption of foods containing calcium, kidney disease, in which the urine excretes more than the required amount of calcium, low levels of magnesium in the blood, which reduces the activity of parathyroid hormones, etc. (Arnbjerg 1980; Mircheva 2006; Toribio 2011; Barrêto-Júnior et al. 2017). Phosphorus levels may increase with impaired renal function due to decreased glomerular filtration rate and as a result of secondary hyperparathyroidism, hypoparathyroidism, hypervitaminosis D (Mircheva 2006).

Low levels of Ca and elevated levels of P were the reason for the observed imbalance between the two macroelements. Nutritional supplements containing Calcium can help regulate blood calcium levels. In addition, it is recommended to take supplements with vitamin D and magnesium.

In the intermediate stage of the experiment, the differences between the breeds within the groups were eliminated. At the end of the experiment, significant differences were observed only in the levels of LDH and Creat, as LDH was higher in the control groups, and Creat was higher in the control group of PAB and in the experimental group of EBB. In the experimental group of the PAB, from the beginning to the end of the experiment the values of Ca and Mon decreased, as their values in the control group increased. GRA, MCHC and RBC remained at the same level in the experimental group and decreased in the control group (Table 3). THR did not change in the experimental group, but increased in the control group. The values of Hct and MCH increased in the experimental group and decreased in the control group. ALT, TBL and Hb decreased from the beginning of the experiment to the end, remaining lower in the

Table 6. Mean values of oestrus duration, sexual cycle and Graafian follicle size in mares of the Purebred Arabian breed (PAB) and East-Bulgarian horse breed (EBB), distributed in groups depending on the diet.

	Duration of oestrus, days		Sexual cycle, days		Size of Graafian follicle, mm SE	
Breeds	Treated	Control	Treated	Control	Treated	Control
Purebred Arabian	4.08 ± 0.30	3.94 ± 0.27	20.20 ± 2.13	21.00 ± 2.46	35.38 ± 1.41	34.89 ± 1.35
Eastbulgarian	3.63 ± 0.38	5.50 ± 0.31	20.00 ± 1.20	22.00 ± 0.93	38.25 ± 1.90	36.94 ± 1.70

control group. P, WBC, RDW and MPV decreased as the values remained lower in the experimental group. Lym values increased from start to finish, remaining higher in the control group, and MCV increased but remained higher in the experimental group.

In the experimental group of the EBB, from the beginning to the end of the experiment the values of Ca, P, Mon, MPV and MCHC decreased in the experimental group and increased in the control group (Table 4). Hct and MCV values increased as the control group values decreased. TBL, WBC, GRA and RDW decreased from the beginning of the experiment to the end, remaining lower in the control group. ALT, RBC, MCH, Hb increased the values in the experimental and decreased in the control group, and in RDW decreased in the experimental and increased in the control group.

Lym and THR values increased from the beginning to the end of the experiment, remaining higher in the control group.

In the duration of the sexual cycle there was a tendency for shortening in both breeds, as in PAB the difference was almost one day (Table 6). In EBB mares, the dietary supplement had a stronger effect, while in the experimental group the sexual cycle was significantly shorter (2 days).

The size of the dominant follicles in both breeds was larger in the experimental group, and again in the case of EBB the difference was more noticeable. Abo-El-Maaty et al. (2013) found significantly larger dominant and subordinate follicles in the Purebred Arabian horses that received an additional mineral supplement in their feed compared to those that received only their main ration. According to the authors, the addition of minerals to the feed improved follicular growth and the development of the corpus luteum.

During the winter season, nutrition is usually associated with limited intake of important vitamins and trace elements, which can lead to deterioration of reproductive performance. Guillaume et al. (2002) in an experiment with restricted mare nutrition found that malnutrition during two consecutive winter periods led to a prolongation of the anoestrus period and a relatively smaller number of sexual cycles for the subsequent mating seasons in the animals in the experimental group. It can be seen that, although the short period of testing of the food supplement showed a tendency to improve the main studied reproductive traits in the mares included in the experimental group.

Conclusion

One week of mineral and vitamin supplementation for 3–4 months could be recommended for mares with low body condition or recovered from any health problem before starting their breeding

In conclusion, the nutritional supplement had a good effect on the reproductive status, and on the blood parameters (hematological and biochemical) of the mares, but more research is necessary to recommend its use.

Ethical approval

The experiment was conducted according to the requirements of Directive 2010/63/EU of the European Parliament and of The Council of 22 September 2010 on the protection of animals used for scientific purposes.

Acknowledgements

This research work was carried out with the support of the Agricultural University–Plovdiv and the Institute of Biology and Immunology of Reproduction in Bulgaria.

Disclosure statement

The authors declare that there is no conflict of interest associated with this paper. The authors alone are responsible for the content and writing of this article.

Funding

This work was financed from the National Scientific Program 'Reproductive Biotechnologies in Animal Breeding in Bulgaria' – REPROBIOTECH funded by The Ministry of Education and Science.

References

Abo-El-Maaty AM, Ibrahim AM, Ezzo OH. 2013. Influence of mineral supplementation on oxidative stress, ovarian follicles growth and reproductive hormone concentration in cyclic Arab mares. Asian Pac J Rep. 2(1):8–14.

Arnbjerg J. 1980. Hypocalcemia in the horse. A case report. Nord Vet Med. 32(5):207–211.

Aros K, Carrasco J, Briones R, Tadich A. 2017. Haematological and serum biochemical reference values for urban-working equines in Chile. Austral J Vet Sci. 49(1):27–33. DOI: https://dx.doi.org/10.4067/s0719-81322017000100027.

Barrêto-Júnior RA, Minervino AH, Rodrigues FA, Meira Júnior EB, Lima A, Sousa R, Mori C, Araújo CA, Fernandes WR, Ortolani E. 2017. Clinical presentation and biochemical profile of horses during induction and treatment of hypocalcemia. Austral J Vet Sci. 49(1):9–14.

Boediker R. 1991. Die bestimmung der gamma-glutamyltransferase (GGT) im serum als indikator fur die kolostralmilch-versorgung des kalbes. Tieraerztl Umsch. 46: 190–194.

Bruck I. 1996. In vitro maturation of equine oocytes: effect of follicular size, cyclic stage and season. Theriogenology. 46(1):75–84.

Cebulj-Kadunc N, Bozic M, Kosec M, Cestnik V. 2002. The influence of age and gender on haematological parameters in Lipizzan horses. J Vet Med Series A. 49(4):217–221.

Gastal EL, Gastal MO, Donadeu FX, Acosta TJ, Beg MA, Ginther OJ. 2007. Temporal relationships among LH, estradiol, and follicle vascularization preceding the first compared with later ovulations during the year in mares. Anim Reprod Sci. 102(3–4):314–321.

Ginther OJ, Gastal MO, Gastal EL, Jacob JC, Beg MA. 2009. Age-related dynamics of follicles and hormones during an induced ovulatory follicular wave in mares. Theriogenology. 71(5):780–788.

Grondin TM, Dewitt SF. 2010. Normal hematology of the horse and donkey. In: Weiss DJ, Wardrop KJ, editors. Schalm's veterinary hematology. Ames (IA): Willey-Blackwell; p. 821–828.

Guillaume D, Duchamp G, Salazar-Ortiz J, Nagy P. 2002. Nutrition in¯uences the winter ovarianinactivity in mares. Theriogenology. 58:593–597.

Gurgoze SY, Icen H. 2010. The influence of age on clinical biochemical parameters in pure-bred Arabian mares. J Equine Vet Sci. 30(10):569–573.

Jain NC. 1993. Comparative hematology of common domestic animals. In: Jain NC, editor. Essentials of veterinary hematology. Philadelphia: Lea and Febiger; p. 19–53.

Kramer JW. 2000. Normal hematology of the horse. In: Feldman BF, Zinkl JG, Jain NC, editors. Schalm's veterinary hematology. Ames (IA): Willey-Blackwell; p. 1069–1074.

Meyer DJ, Harvey JW. 2004. Veterinary laboratory medicine: interpretation and diagnosis. 3rd ed. St. Louis (MO): Saunders.

Mircheva T. 2006. Basics of clinical biochemistry in domestic animals. 1st ed. IK Enyovche. 94.

Morel MC, Newcombe JR, Hayward K. 2010. Factors affecting pre-ovulatory follicle diameter in the mare: the effect of mare age, season and presence of other ovulatory follicles (multiple ovulation). Theriogenology. 74 (7) :1241–1247.

Toribio RE. 2011. Disorders of calcium and phosphate metabolism in horses. Vet Clin North Am Equine Pract. 27(1): 129–147.

Padilha FGF, Dimache LAG, Almeida FQ, Ferreira AMR. 2017. Blood biochemical parameters of Brazilian sport horses under training in tropical climate. R Bras Zootec. 46(8): 678–682.

Popov V, Yorov I, Dzhurkov D, Zhelyazkov S. 1974. Testing the effect of compound feedingstuffs on purebred Arabian mares feeding during pregnancy and lactation. Anim Sci. XI(8):135–138.

Popova M. 2017. Condition, dynamics and development trends of breeding and reproductive status of horses from Purebred Arabian and Shagya breeds. Dissertation. Plovdiv: Agricultural University; p. 225. (Bg)

Sabeva I, Popova M. 2019. Monitoring of genealogical structure development of Eastbulgarian breed horses originating from stallions of other sporting breeds. AST. 11(3): 195–197.

Sabeva I, Popova M, Kastchiev S. 2018. Sources of specific variance and heritability of free jump qualities in 2-years old horses from the East Bulgarian breed. Bulg J Agric Sci. 24 (Suppl. 2):85–89.

Winnicka A. 2011. Reference values of basic laboratory tests in veterinary medicine. 5th ed. Warszawa (Poland): SGGW; p. 136.

Yur F, Dede S, Deger Y, Kilicalp D. 2008. Effects of vitamin E and selenium on serum trace and major elements in horses. Biol Trace Elem Res. 125(3):223–228.