



Original Contribution

**CHANGES IN LEVELS OF SOME INNATE IMMUNE RESPONSE
PARAMETERS, BLOOD VITAMINS E AND A IN STALLIONS,
FOLLOWING CASTRATION**

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ABSTRACT

We examined in stallions the impact of castration on total differential count of leukocytes and the classical pathway of complement activation (CPCA), blood vitamins A and E levels. The stallions were castrated with an emasculator, while in standing position, using the open technique. The number of leukocytes was counted in the Burker chamber, and the leukogram on blood smear. The neutrophil/lymphocyte ratio (N/L) was counted; CPCA was shown with Mayer's method adapted for horses. Vitamins E and A concentrations were determined spectrophotometrically. The castration led to an increase in neutrophil bands number 3 hours later and on days 3 and 7; monocytes band number increased also 3 hours post castration. The N/L ratio increased on the 24th hour. The CPCA was reduced on the 0 hour, and the amount of vitamin E - on the 24th hour. The applied castration caused temporary and quantitative changes in the examined parameters of innate defence by suppressing its humoral component – complement (0 hour) and caused an increase of the cell elements – neutrophils-band and monocytes and the N/L ratio. It led to E hypovitaminosis.

Key words: castration, innate immunity, vitamin A, vitamin E, stallions

INTRODUCTION

Surgical impact and post-operational pain are stress factors which demand neuroendocrine and tissue answers. The latter induce changes in the immune defence (1, 2), in metabolism, cardiovascular and inflammatory mechanisms (3).

Castration in horses, viewed as common and routine surgical procedure involves a high degree risk of complications (4). The first barrier of defence, initiated by this surgical trauma includes the elements of natural resistance – complement, phagocytes, acute phase proteins, cytokines and others. After castration Fisher et al. (5) observed neutrophil leukocytosis and a high concentration of plasma haptoglobin and fibrinogen, whereas Okumura et al. (6) – a high concentration only of haptoglobin.

There are scarce data about the fact that some kinds of stress induce vitamin E deficiency (VE) (7, 8), and none about the effect of castration. Besides, Miller et al. (9) confirmed that in hypovitaminosis A and E the specific and non-specific immune mechanisms were suppressed. Hogan et al. (10) found reduction of phagocyte function in neutrophils, which increased the risk of bacterial infections. The effects of treating with VE and vitamin A (VA) on the superoxidant production is correlated with changes in intracellular signalling of neutrophils and by an increase in their phagocyte and killing activity (11).

The aim of the current research was to study the dynamics of changes, brought about by castration, on complement and the white blood cells as non-specific factors of defence, and in the VE and VA levels, in stallions.

MATERIALS AND METHODS

Animals

Four healthy Hannover bred stallions, obtained from the Experimental Equine Base

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of Trakia University – Stara Zagora, Bulgaria, were used. They were 4-5 years of age, with body weight of 500-600 kg. The horses were housed in box stalls under ambient light conditions and fed a diet of commercially available pellets and mixed alfalfa-grass hay. Salt and water were available *ad libitum*.

Castration

The castration was performed in standing patient position by the open method using an emasculator. The following anaesthesia was used: 2% xylazine (as HCl) (*Alfasan, Woerden - Holland*); it was injected into the vein at a dose of 1 mg/kg body weight. After 10 min, 2% Morphium hydrochloricum (*Sofarma Ltd – Sofia*), at 0.5mg/kg body weight, was injected also into the vein. Prior to the testicular incision, 15 ml of a 0.5 % solution of Novocain (*Vetprom Ltd, Radomir, Bulgaria*) was injected into the testicles.

Blood samples

Blood samples were taken from *v. jugularis externa* before castration, immediately after (0 h), and subsequently on the 3rd and 24th hour, 3rd, 7th and 14th day after castration. The samples were either centrifuged (4°C) within 15 min of collection to obtain the plasma, or allowed to clot at 20 to 22°C prior to centrifugation to obtain the serum.

Parameters of the natural immune response

The total number of leukocytes was counted in the Bürker chamber, the leukogram was determined by microscopic observation of blood smears, stained with May-Grunwald-Giemsa dyes, using the Meandre counting method. The classical pathway complement activation (CPCA) was established according to the method of Mayer (12), adapted for horses (13).

Neutrophil-lymphocyte (N/L) ratio

To estimate the stress of castration the N/L ratio was used (14). It was calculated by dividing the number of neutrophils by the number of lymphocytes.

Vitamins

The vitamin A content in blood serum was determined by the method of Dest as described by Schneider and Widmann (15), and of VE – in blood plasma following the procedure of Spiritchef et al. (16).

Statistical Analysis

The results were expressed as mean \pm SEM and subjected to statistical analysis of variance (ANOVA). Differences were considered statistically significant at the $p < 0.05$ level.

RESULTS

The data on the investigated parameters are shown on **Table 1**. The total number of leukocytes and the number of segmented neutrophils have a trend of increase to maximum on the 3rd h, with no significant value. An increase in the number of neutrophil bands versus initial level was observed best on the 3rd h ($p < 0.01$), 3rd day ($p < 0.05$) and 7th day ($p < 0.01$). Similar changes were registered for monocytes on the 3rd h ($p < 0.05$). A tendency towards decrease was evident in eosinophils – on 3rd h and in lymphocytes – on 24th h, with a lack of significant differences from initial values.

N/L ratio increased gradually, and was highest on the 24th h ($p < 0.05$), after which it gradually decreased with time.

CPCA activity decreased immediately after castration ($p < 0.05$). On the 24th h it returned back to normal values.

The concentration of VE decreased most prominently on 24th h ($p < 0.05$), and returned to original values between the 7th and 14th day. In contrast, VA tended to increase gradually up to 24th h and between 3rd and 14th day; a reversal towards initial values was observed.

DISCUSSION

The application of surgical interference (castration) caused significant changes in the non-specific defence mechanisms, as well as in the amount of vitamins. The increase of N/L ratio, observed on 24th h ($p < 0.05$) indicated that horses may have undergone stress. Such ratio has been found to be a definite indicator of stress in horses (17, 18) whereas between the ratio and the cortisol concentration a positive correlation has been set to take place [19]. The neutrophil content as an important part of the non-specific cell defence after applied castration was determined by a non-significant segmented neutrophilia and significant increase of band neutrophils on the background of feebly expressed leukocytosis. An increase in the total counts of leukocytes and neutrophils after castration has been determined by Kelani and Durotoye (20) and Fischer et al. (5). Possible reasons for these changes may be related on the one hand to the fact that

perhaps after castration the cortisol level increased and caused mobilization of band neutrophils from the bone marrow (left shift). It is possible that this left shift could be due to tissue damage after surgical procedure as an expression of inflammatory response. The observed neutrophilia was accompanied by monocytosis on the 3rd h ($p < 0.05$). For now it

is not well understood why there is an increase in monocyte counts after castration. Even so, monocytes are similar to neutrophils and are a part of the natural resistance, activated under the influence of the surgical operation and the accompanying significant trauma.

Table 1: Changes in total and differential count of leukocytes, neutrophil/lymphocyte ratio (N/L), classical pathway of complement activity (CPCA), concentrations of vitamin E (VE) and vitamin A (VA) before and after castration in stallions ($n=4$). Values are presented as mean \pm SEM. Level of significance ^a $p < 0,05$; ^b $p < 0,01$ versus initial level.

Parameters	Before	0 hr	3 hr	24 hr	3 day	7 day	14 day
Leukocytes ($\times 10^9/l$)	6.68 \pm 0.68	6.68 \pm 0.8	8.8 \pm 0.22	7.73 \pm 0.99	7.38 \pm 1.21	7.48 \pm 0.33	7.18 \pm 1.13
Neutrophils-bands ($\times 10^9/l$)	0.12 \pm 0.02	0.13 \pm 0.06	0.43 \pm 0.13 ^b	0.27 \pm 0.06	0.41 \pm 0.08 ^a	0.44 \pm 0.07 ^b	0.22 \pm 0.06
Neutrophils – segm. ($\times 10^9/l$)	3.53 \pm 0.57	3.93 \pm 0.55	5.29 \pm 0.39	5.1 \pm 0.79	3.5 \pm 0.68	4.02 \pm 0.39	4.24 \pm 0.89
Eosinophils ($\times 10^9/l$)	0.17 \pm 0.04	0.09 \pm 0.04	0.07 \pm 0.02	0.12 \pm 0.03	0.24 \pm 0.08	0.24 \pm 0.05	0.22 \pm 0.06
Monocytes ($\times 10^9/l$)	0.08 \pm 0.03	0.11 \pm 0.03	0.29 \pm 0.10 ^a	0.25 \pm 0.09	0.22 \pm 0.05	0.29 \pm 0.09	0.24 \pm 0.04
Lymphocytes ($\times 10^9/l$)	2.77 \pm 0.31	2.43 \pm 0.48	2.57 \pm 0.48	1.99 \pm 0.42	2.68 \pm 0.45	2.48 \pm 0.54	2.48 \pm 0.42
N/L ratio	1.32 \pm 0.26	1.83 \pm 0.39	2.32 \pm 0.48	3.03 \pm 0.78 ^a	1.30 \pm 0.08	1.99 \pm 0.59	1.70 \pm 0.18
CPCA (CH 50)	20.6 \pm 0.9	18.4 \pm 0.48 ^a	19.3 \pm 0.38	20.3 \pm 0.68	20.5 \pm 0.51	19.01 \pm 0.45	18.9 \pm 0.2
VE ($\mu\text{mol/l}$)	70.8 \pm 11.3	59.6 \pm 13.1	55.5 \pm 15.1	33.8 \pm 4.26 ^a	57.4 \pm 5.2	75.3 \pm 15.5	78.3 \pm 15.0
VA ($\mu\text{mol/l}$)	1.13 \pm 0.18	1.39 \pm 0.26	1.55 \pm 0.25	1.56 \pm 0.05	1.17 \pm 0.17	1.15 \pm 0.19	1.15 \pm 0.09

The tendency towards a decrease of the number of lymphocytes, especially after the 24th h following the castration was probably a result of immune suppression, originating from the hypersecretion of glucocorticoids (21).

The castration of horses was accompanied by a decrease in the level of CPCA from 0 h. This probably was due to the inhibiting action of glucocorticoids. According to Kapsala et al. (22) these hormones suppress production, secretion and activation of complement.

After castration the concentration of VE on the 24th h decreased. Similar to our results are the data published by other authors (7), which have investigated other stress factors. Madsen F. (23) found out that during stress the organism's demand for some antioxidants increased, including VE. This vitamin neutralizes the toxic products of free radical oxidation, which are increased in stressful conditions, where the enforced process of peroxidation lowers the amount of antioxidant VE (24).

This study shows that castration was experienced as a stress factor (N/L ratio is increased on 24th h), that initiated segmented neutrophilia (on 3rd h and from 3rd up to 7th day), monocytosis (on 3rd h), hypovitaminosis E (on 24th h) and decrease in CPCA values (0 h).

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