

VETERINARSKI ARHIV 75 (3), 195-202, 2005

Concentrations of total proteins and albumins, and AST, ALT and GGT activities in the blood plasma of mares during pregnancy and early lactation

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MILINKOVIĆ-TUR, S., V. PERIĆ, Z. STOJEVIĆ, M. ZDELAR-TUK, J. PIRŠLJIN:
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195-202, 2005.

ABSTRACT

The metabolism of proteins in the blood plasma of Holstein breed mares was investigated during pregnancy and early lactation. Blood for analysis was taken in the first, second and third periods of pregnancy, and during early lactation. Blood samples were collected by jugular puncture with heparin as an anticoagulant. In the obtained blood plasma the concentration of total proteins and albumin, and the activity of enzymes aspartate aminotransferase (AST; EC 2.6.1.1), alanine aminotransferase (ALT; EC 2.6.1.2), and gamma-glutamyltransferase (GGT; EC 2.3.2.2) was determined by spectrophotometry. According to the research results of the blood plasma in mares during pregnancy and early lactation, there were no significant differences in the concentration of total proteins, albumin, and ALT and GGT enzyme activity. In the final third of pregnancy there was a statistically significant decrease of AST activity compared to the first period ($P < 0.01$), and the second period of pregnancy ($P < 0.001$). The lower activity of AST during pregnancy and early lactation can be attributed to this special breed. The obtained results constitute a contribution to a better understanding of biochemical processes in pregnant mares, for estimating their physiological status and for diagnostic purposes.

Key words: mares, pregnancy, early lactation, total plasma proteins, albumins, transferase

Introduction

Blood plasma contains three major protein fractions: albumin, globulin, and fibrinogen. In humans, sheep, goat, rabbits and rat there are mostly albumins, while in horse, pig

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and cattle the ratio of albumins and globulins is almost equal, or globulins prevail (FORENBACHER, 1993; SWENSON, 1993).

Aspartate aminotransferase (AST) is a widely distributed enzyme, which is found in many tissues and organs, with high activity in the liver (ZIMMERMAN et al., 1968). Increased AST activity in the serum is a sensitive marker of liver damage (MEYER and HARVEY, 1998). There are two main isoenzymes: mitochondrial and cytosolic, which prevails in the total concentration in the blood plasma because it has a longer half-life (KRAMER and HOFFMAN, 1997). Activity of AST in horses is much higher than in other animals (CORNELIUS et al., 1958). In Hafling horses the activity of serum AST amounted 146.8 ± 5.6 U/L (WEIGERT et al., 1980), while KANEKO et al. (1997) mention values of 296 ± 70 U/L. In addition to species, breed and age, AST activity is influenced by muscle activity (WEIGERT et al., 1980). Working horses have an approximately 60% higher activity (112 IU/L) than horses which are at rest for several days (70 IU/L) (WEIGERT et al., 1980).

In primates, dog, cat, rabbit and rat, alanine aminotransferase (ALT) is a specific cytosol liver enzyme, and its increase in the blood plasma is specific for changes in the liver, but ALT activity in pigs, horses, goats, sheep and cattle is not specific for the liver, in order to have a diagnostic significance (KRAMER and HOFFMAN, 1997). ALT activity in the blood plasma is influenced by age and muscle activity (WEIGERT et al., 1980).

Gamma-glutamyltransferase (GGT) is a membrane-bound enzyme that is found in cells with high rates of secretion or absorption. The high activity is found in the liver, kidneys, pancreas, intestine and the spleen (TENNANT, 1997). In the livers of cow, horse, sheep, and goat the GGT activity is relatively high, and considerably lower in dog and cat. Although GGT activity is present in many tissues, it is considered a serum marker primarily for the diagnosis of liver disease in animals. According to some authors the enzyme is tied to the metabolism of glutathione, which has an important role in the entire antioxidative status of the organism (KRAMER and HOFFMAN, 1997).

The aim of this research was to investigate the metabolism of proteins and the activities of mentioned enzymes in the blood plasma during pregnancy and early lactation in valuable Holstein mares. Because there are no systematic data for those important parameters of the metabolic status for these animals, the obtained results may represent a contribution to a better understanding of biochemical processes in pregnant Holstein mares, as well as for estimating their physiological status, and for diagnostic purposes.

Materials and methods

The experiment was performed on six Holstein mares aged from 5 to 10 years, monitored during pregnancy and early lactation. The animals were held in stables, with occasional outdoor stays in spring and summer. They were fed twice a day with hay and

oats, according to the period of pregnancy and lactation, and with vitamin and mineral supplements. The blood for analysis was taken three times during pregnancy and during early lactation. In the first group (n = 6) were mares up to the 16th week of pregnancy, in the second group (n = 6) were mares from the 17th to the 31st week, and in the third group (n = 6) mares from the 32nd until the 48th week of pregnancy. The fourth group (n = 6) consisted of mares from the 1st to the 14th week of lactation.

Blood samples were collected by puncture of the v. jugularis, with the addition of heparin as an anticoagulant. The blood plasma was separated by centrifugation at 1500 g for 10 minutes. The concentration of total plasma proteins and albumins, together with the activities of aspartate aminotransferase (AST; EC 2.6.1.1), alanine aminotransferase (ALT; EC 2.6.1.2), and gamma-glutamyltransferase (GGT; EC 2.3.2.2) in the blood plasma, were determined spectrophotometrically on a Helios gamma UV visible spectrophotometer, Thermo spectronic UK, by commercial sets from "Herbos dijagnostika d.o.o.", Sisak, Croatia.

The obtained results were treated by statistical calculations of the mean value, standard deviations, standard errors of the mean value and variability coefficient. The significant difference among results was checked by Student's *t*-test, and statistical significance was taken to be indicated by $P < 0.05$.

Results

Concentrations of total plasma proteins and albumins, as well as enzyme AST, ALT and GGT activity in the blood plasma of mares during pregnancy and early lactation are shown in Figs 1 and 2.

In the first 16 weeks of pregnancy (first third of pregnancy) the concentration of total proteins in mare blood plasma was approximately 67.40 ± 0.96 g/L. In mares from the 17th to the 31st week of pregnancy (second third of pregnancy) the concentration of total plasma proteins increased to approximately 69.29 ± 0.24 g/L. In mares from the 32nd until the 48 week of pregnancy (final third of pregnancy) the concentration of total plasma proteins averaged 68.73 ± 1.12 g/L, while during early lactation the average concentration was 62.46 ± 5.17 g/L (Fig. 1). By comparing the obtained results statistically significant differences were not recorded.

Concentration of albumins in the blood plasma during the first third of pregnancy was 33.13 ± 1.81 g/L, and in the second third of pregnancy 31.48 ± 1.62 g/L. In the final third of pregnancy, from the 32nd to the 48th week, the concentration of albumins increased and averaged 42.18 ± 5.59 g/L. During early lactation the concentration of albumins averaged 36.67 ± 1.96 g/L (Fig. 1). By comparing the obtained results, statistically significant differences were not recorded.

AST enzyme activity in the blood plasma during the first third of pregnancy was 126.88 ± 8.22 U/L. In the second third of pregnancy AST activity averaged 124.15 ± 4.43 U/L. During the final third of pregnancy, decrease of AST activity in mare blood plasma was recorded, averaging 97.00 ± 2.94 U/L. During early lactation AST activity averaged 117.27 ± 8.50 U/L (Fig. 2). By comparing the obtained results, a statistically significant decrease was recorded in the final third of the pregnancy period relating to the first third on the level of significance $P < 0.01$; and to the second third of pregnancy at $P < 0.001$.

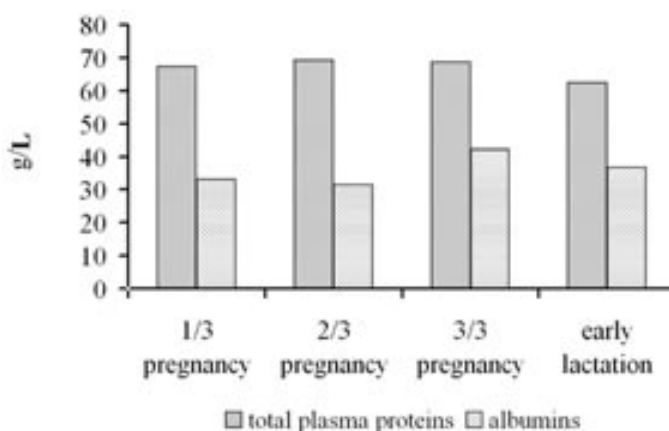


Fig. 1. Concentrations of total proteins and albumins in the blood plasma of mares during pregnancy and early lactation, expressed in g/L.

During the first third of pregnancy the activity of ALT in the blood plasma averaged 3.69 ± 0.63 U/L, and in the second third of pregnancy 4.08 ± 0.26 U/L, while in the final third of pregnancy enzyme activity averaged 3.97 ± 0.85 U/L (Fig. 2). ALT enzyme activity during early lactation averaged 5.63 ± 1.40 U/L (Fig. 2). During the entire experimental period ALT activity in the blood plasma was at nearly the same level and by comparing the results statistically significant differences were not recorded.

During the first 16 weeks of pregnancy, GGT enzyme activity averaged 10.55 ± 1.48 U/L, in the second third of pregnancy it averaged 10.19 ± 0.93 U/L, in the final third of the pregnancy period it was 8.67 ± 0.71 U/L. During early lactation GGT enzyme activity averaged 9.99 ± 1.61 U/L (Fig. 2). By comparing the obtained results, statistically significant differences were not recorded.

Discussion

Pregnancy and lactation have a great impact on the intensity of metabolism and on metabolic parameters in the blood. The activity of aminotransferases in blood is very important because they act as a catalyst in connection with the metabolism of amino acids and carbohydrates. Changes in their activity in the blood can be a consequence of their increased activity in the cells, or cell structure damage.

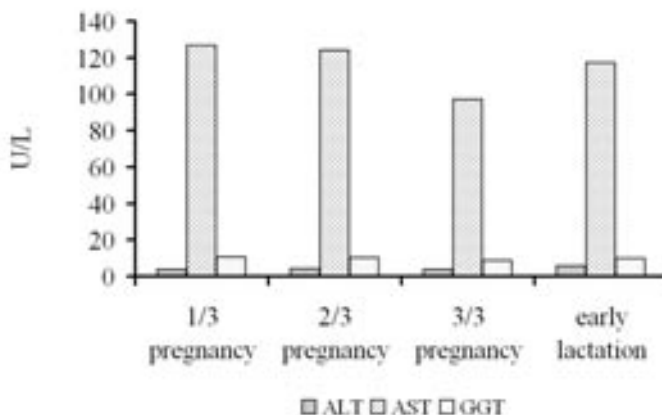


Fig. 2. Activity of AST, ALT and GGT in the blood plasma of mares during pregnancy and early lactation, expressed in U/L. The significance of differences on the level * $P < 0.01$; ** $P < 0.001$.

The concentration of total proteins in mare blood plasma during pregnancy is within physiological values (FORENBACHER, 1993). According to the results of this study, the concentration of total proteins in mare blood plasma during pregnancy and in early lactation did not alter significantly (Fig. 1). The slight increase in total protein concentrations in the second and third periods of pregnancy, compared to the beginning of pregnancy, is a result of hormonal changes in the organism. The secretion of other hormones (glucocorticoids, thyroxin) increases during pregnancy as well, as a consequence of increased sex hormone secretion, which in turn intensifies metabolic events in the organism (NETT et al., 1973; CUNNINGHAM, 1997). Glucocorticoids improve the mobilization of extrahepatic proteins and transport of amino acids to liver cells. Mobilized amino acids in liver cells will help synthesize glucose through gluconeogenesis, which is the primal source of energy for the embryo. The gradual increase of total protein concentration in the blood plasma of mares during pregnancy was recorded by HERAK et al. (1994).

The values of albumin concentration in the first and second thirds of the pregnancy period were similar (Fig. 1), and the obtained results are in agreement with those reported by SWENSON (1993). The highest concentration of albumins was measured in the final

third of the pregnancy period, which is actually the time when an abundant synthesis of proteins in the liver occurs.

The research results of ALT and GGT enzyme activities in the pregnant mares' blood plasma, and during early lactation, did not alter significantly (Fig. 2). As can be seen from the presented results, GGT activity decreased slightly during pregnancy, and in the final third of the pregnancy period it reached its lowest value. KANEKO et al. (1997) report GGT values for horses between 4.3 and 13.4 U/L, which means that GGT activity in this research remains within physiological values.

Given that AST activity is highest in ungulates (KRAMER and HOFFMAN, 1997), amounting from 146.8 U/L (FORENBACHER, 1972) to 286 U/L (KANEKO et al., 1997), the data obtained in this research show a reduced AST activity in the blood plasma of Holstein mares during pregnancy and early lactation. The value span of most serum enzymes in physiological conditions depends on age, breed, gender, pregnancy, breast-feeding, feeding, holding conditions and exploitation (GERBER et al., 1973). However, with all the mentioned factors large individual deviations were recorded. Since there is no information about Holstein mares, and as it is known that AST activity varies depending upon the breed, we are unable to state with certainty that the obtained values are beneath physiological limits. A significant reduction of AST activity was determined in the final third of the pregnancy period compared to the first ($P < 0.01$) and the second ($P < 0.001$) thirds, which is consonant with the results of HERAK and HERAK (1966), who recorded the lowest serum AST activity in the final third of the pregnancy period. A slight increase of AST activity in the period of early lactation can be attributed to glucocorticoid action during birth, which gradually decreases during lactation.

According to the research results, concentrations of total plasma proteins and albumins, and ALT and GGT enzyme activity in the blood plasma of Holstein mares during pregnancy and early lactation, remained within the known values recorded in different breeds. The lower activity of AST enzymes during pregnancy and early lactation can be attributed to this special breed. The obtained results could serve for a better understanding of biochemical processes in pregnant mares, for estimating their physiological status and for diagnostic purposes.

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Received: 30 January 2004

Accepted: 5 May 2005

MILINKOVIĆ-TUR, S., V. PERIĆ, Z. STOJEVIĆ, M. ZDELAR-TUK, J. PIRŠLJIN: Kretanje koncentracija ukupnih bjelančevina i albumina te aktivnosti AST, ALT i GGT u krvnoj plazmi kobila tijekom gravidnosti i rane laktacije. Vet. arhiv 75, 195-202, 2005.

SAŽETAK

Istražen je metabolizam bjelančevina u krvnoj plazmi kobila pasmine Holstein tijekom gravidnosti i rane laktacije. Krv za analizu uzimana je u prvoj, drugoj i trećoj trećini gravidnosti, te u ranoj laktaciji iz v. iugularis uz upotrebu heparina kao antikoagulansa. U dobivenoj krvnoj plazmi spektrofotometrijski je određena koncentracija ukupnih bjelančevina i albumina te aktivnosti enzima aspartat aminotransferaze (AST; EC 2.6.1.1.), alanin aminotransferaze (ALT; EC 2.6.1.2.), i gama-glutamilttransferaze (GGT; EC 2.3.2.2.). Na osnovi polučenenih rezultata u krvnoj plazmi kobila tijekom gravidnosti i rane laktacije nisu utvrđene značajnije promjene koncentracija ukupnih bjelančevina i albumina te aktivnosti enzima ALT i GGT. U posljednjoj trećini gravidnosti utvrđen je statistički značajan pad aktivnosti AST u odnosu na prvu ($P<0,01$) i drugu trećinu ($P<0,001$). Niža aktivnost AST tijekom gravidnosti i rane laktacije mogla bi se pripisati pasminskoj osobitosti. Dobiveni rezultati doprinose boljem poznavanju biokemijskih procesa u gravidnih kobila, kako u procjeni njihovog fiziološkog stanja tako i u dijagnostičke svrhe.

Ključne riječi: kobile, gravidnost, rana laktacija, ukupne bjelančevine plazme, albumini, transferaze
