

Blood serum proteinograms in pregnant and non-pregnant cows

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ABSTRACT

The proteinograms of non-pregnant and pregnant cows were studied. Significant differences in total protein, albumin, globulin, α -globulin and β -globulin concentrations ($P < 0.01$) between non-pregnant and pregnant cows during the eighth month of pregnancy were recorded. The differences in concentrations of γ -globulin and in the A:G ratio were not significant ($P > 0.05$). The comparison of results recorded in the eighth and in the ninth months of pregnancy showed that the globulin, α -globulin and β -globulin concentration significantly decreased in the ninth month of pregnancy ($P < 0.01$), the concentration of total serum protein also decreased significantly ($P 0.01-0.05$), albumin concentration ($P > 0.05$) and A:G ratio ($P > 0.01$) significantly increased, whereas in γ -globulin concentration the difference between the values recorded in the eighth and in the ninth months of pregnancy was not significant ($P > 0.05$).

Key words: proteinogram, gravidity, pregnancy, electrophoresis, cattle

Introduction

During a research into proteinograms of clinically healthy cattle it was noticed that the proteinograms of gravid cows are conspicuous for their increased concentrations of total serum proteins, total globulin content and α -globulin fraction, a fact that is also recorded in the literature (LIBERG, 1977) as well as for the concentration of γ -globulin, which, however, is not recorded in literature. Since the number of gravid

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cows in the sample (N=15) was relatively low, it was necessary to repeat the same research on a larger sample of gravid cows. Pregnancy significantly influences the proteinogram of serum proteins in all animal species (KANEKO, 1989; JAIN, 1993; JEFFCOTT, 1974; TRUMEL et al., 1996; YOSHIDA, 1986). Proteinograms recorded during pregnancy were best researched in mares, but to a lesser extent in cows. During pregnancy there is a tendency for an albumin concentration decrease on the one hand, and a globulin concentration increase on the other (KANEKO, 1989). In mares, the amount of total serum proteins increases up to two or three weeks prior to foaling, when a sudden decrease occurs, which then gradually continues and reaches its lowest point two weeks after foaling (JEFFCOTT, 1974). Albumin concentration decreases and reaches its lowest point in the middle of pregnancy; it then gradually increases to a point where it is within the normal value limits, where it remains until the moment of foaling (KANEKO, 1989). The γ -globulin concentration in the serum of gravid mares starts to increase about three weeks prior to foaling; it then suddenly decreases and reaches its lowest point three weeks after foaling, following which a sudden increase occurs (JEFFCOTT, 1974). In gravid cows the concentration of total serum proteins starts to increase two months prior to calving, reaching its maximum a month prior to calving; it then suddenly decreases as the moment of calving approaches (KANEKO, 1989). The γ -globulin concentration increases until the eighth month of pregnancy and then starts to decrease due to the concentration of immunoglobulin in the mammary gland (MCGUIRE and ADAMS, 1982); it suddenly decreases immediately prior to calving, increasing again during the first weeks after calving (DIXON et al., 1961).

Materials and methods

The research was carried out on 60 clinically healthy cross Simmental breed cows aged 1-7 years. The animals were kept on various family farms around Zagreb, Croatia (Mala Gorica, Gornja Jelenska, Potok, Popovača). Of this number, 50 cows were in various stages of pregnancy. The remaining 10 cows were not gravid. The animals were divided into seven groups. Group one comprised non-gravid cows, group two comprised cows in the 4th month of pregnancy, those in group three were 5 months pregnant, those in group four were 6 months pregnant, those in group five were 7 months pregnant, those in group six were 8 months pregnant, while those in group seven were in their 9th month of pregnancy.

Blood samples were taken from v. jugularis externa into test tubes without an anticoagulant. The samples were kept at room temperature for

2-5 hours to coagulate and were then centrifuged at $1,200\times g$ for 10 minutes. The serum was then separated.

Total protein and albumin concentrations in the serum, as well as the A:G ratio, were determined in each sample, and electrophoresis was carried out.

Concentration of total serum proteins was determined in each serum sample by means of the biuret method (GREEN et al., 1982) on a biochemical autoanalyzer Technicon RA 1000 (Technicon Instruments Corporation, New York, USA), while the serum albumin concentration was determined by means of the bromine-cresol-green (BCG) method (BUSH, 1998) on a biochemical autoanalyzer Technicon RA 1000 using chemicals supplied by Randox (Randox Laboratories LTD., United Kingdom). The globulin concentration in the serum and the A:G ratio were calculated from the known total protein and albumin concentration in the serum.

Electrophoresis was carried out at 200 V for 30 minutes on cellulose acetate strips (Cellogel®, Chemetron, Milan, Italy). A barbitone buffer was used (Barbitone buffer 0.057 mol/l, pH 8.6, ionic strength 0.05, 10.3 g potassium diethylbarbiturate ($C_8H_{11}N_2NaO_3$, Mr 206.18) and 1.34 g of diethylbarbituric acid ($C_8H_{11}N_2NaO_3$, Mr 184.20)) was dissolved in 1 l of distilled water (Veronal®, Kemika, Zagreb, Croatia). The strips were coloured with Ponceau S colour (Ponceau S, Chemetron, Milan, Italy), washed out in 5% acetic acid 3-4 times, each time for five minutes, and immersed in methanol for 30 seconds to dehydrate. The strips were then immersed for one minute in a solution consisting of two parts of glacial acetic acid and eight parts of methanol in order to achieve transience. They were then dried at 95-100 °C (BARTA and ARNOLD, 1993). Protein fractions relationship was read off a densitometer and the absolute concentration of individual fractions in g/l was calculated from the percentages obtained by a densitometer, and the amount of total proteins in the sample determined on an autoanalyzer Technicon RA 1000.

The Student's t-test (VAN DEN BROEK, 1992) was used for statistical data processing; the data for which P was less than or equal to 0.01 were considered to be very significantly different, whereas the data for which P was between 0.01 - 0.05 were considered to be significantly different; data for which P was more than 0.05 were not considered to be significantly different.

Results

The concentration values of total protein, albumin, globulin, certain globulin fractions and A:G ratio in non-pregnant cows and in the four-, five-, six-, seven-, eight- and nine-month pregnant cows are presented in Table 1. The results are presented as mean \pm standard deviation (SD).

Table 1. Concentrations of total proteins and individual protein fractions in the serum of non-pregnant cows and pregnant cows (mean \pm SD)

Month of pregnancy	N	Total proteins (g/l)	Albumin (g/l)	Globulin (g/l)	(g/l)	(g/l)	(g/l)	A:G
non-pregnant	10	65.6 \pm 1.32	29.0 \pm 0.40	36.6 \pm 1.40	7.46 \pm 0.55	8.18 \pm 0.42	19.61 \pm 1.71	0.8 \pm 0.04
4	5	72.0 \pm 1.60	32.4 \pm 3.50	39.6 \pm 3.68	8.73 \pm 0.20	8.54 \pm 0.89	22.37 \pm 4.02	0.8 \pm 0.14
5	5	70.2 \pm 2.96	32.0 \pm 2.00	38.2 \pm 3.52	8.15 \pm 1.57	6.21 \pm 0.94	20.94 \pm 2.67	0.9 \pm 0.12
6	10	71.1 \pm 3.12	33.3 \pm 1.70	37.8 \pm 3.68	9.33 \pm 0.93	6.65 \pm 1.08	19.69 \pm 2.85	0.9 \pm 0.13
7	10	74.8 \pm 3.48	30.2 \pm 1.56	42.6 \pm 5.60	11.56 \pm 1.96	7.74 \pm 1.05	24.23 \pm 3.44	0.7 \pm 0.11
8	10	75.5 \pm 4.50	31.5 \pm 1.70	44.0 \pm 5.20	12.34 \pm 2.57	8.58 \pm 1.05	23.49 \pm 3.53	0.7 \pm 0.12
9	10	69.0 \pm 3.80	32.0 \pm 2.20	36.5 \pm 3.70	7.61 \pm 0.81	7.74 \pm 1.57	19.22 \pm 2.40	0.9 \pm 0.12
KANeko (1989)	Range	67.4-74.6	30.3-35.5	32.4-42.5	7.5-8.8	8.0-11.2	16.9-22.5	0.84-0.94
	Mean \pm SD	71 \pm 1.8	32.9 \pm 1.3	37.2 \pm 1.4	7.9 \pm 0.2	9.6 \pm 0.8	19.7 \pm 1.4	0.89 \pm 0.05

Table 2. Concentrations of total proteins and individual protein fractions in the serum of non-pregnant cows and cows in the eighth and ninth months of pregnancy (mean \pm SD)

Parameter	Non-pregnant	8 th month of pregnancy
Total protein (g/l)	65.6 \pm 1.32	75.5 \pm 4.50 ^a
Albumin (g/l)	29.0 \pm 0.40	31.5 \pm 1.70 ^a
Globulin (g/l)	36.6 \pm 1.40	44.0 \pm 5.20 ^a
-globulin (g/l)	7.46 \pm 0.55	12.34 \pm 2.57 ^a
-globulin (g/l)	8.18 \pm 0.42	8.58 \pm 1.05 ^c
-globulin (g/l)	19.61 \pm 1.71	23.49 \pm 3.53 ^a
A:G ratio	0.8 \pm 0.04	0.7 \pm 0.12 ^c

^a=P<0.01 very significant; ^b=P<0.05 significant; ^c=P>0.05 non significant

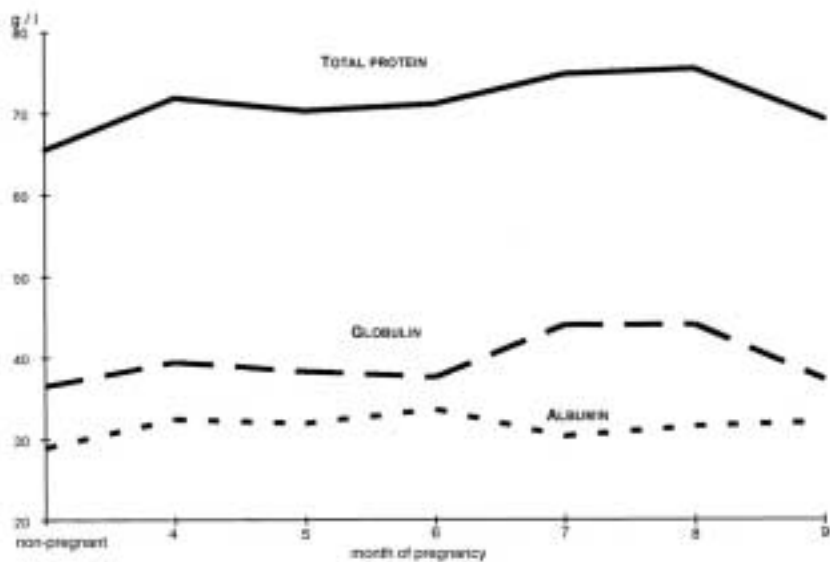


Fig. 1. Total protein, albumin and globulin concentration in serum of non-pregnant cows and cows during various stages of pregnancy

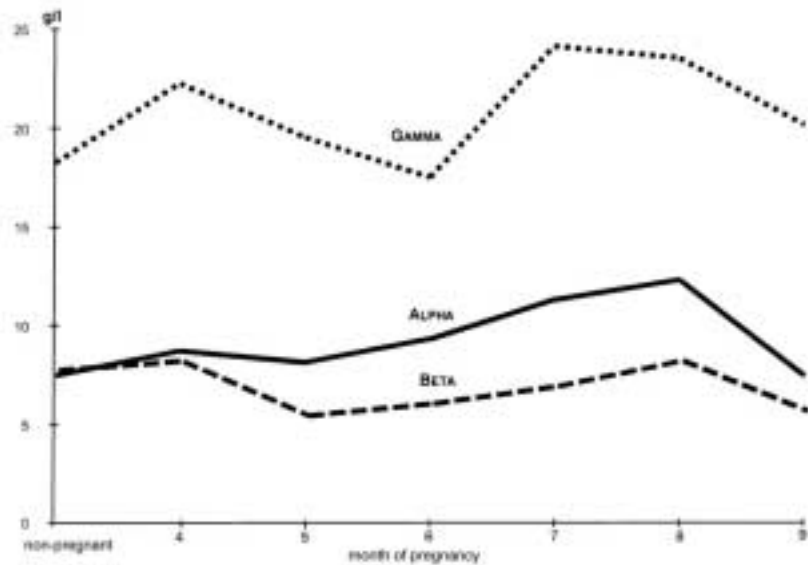


Fig. 2. Serum alpha, beta and gamma globulin concentration in non-pregnant cows and cows during various stages of pregnancy

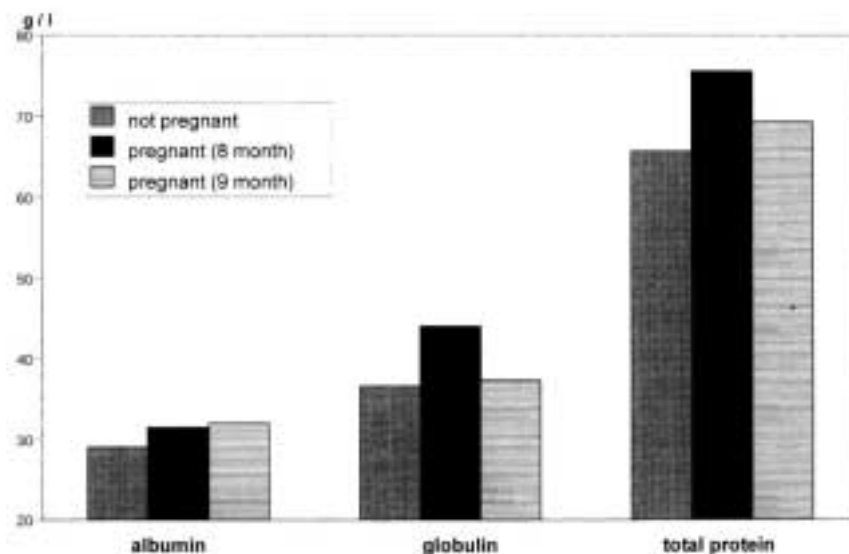


Fig. 3. Serum total protein, albumin and globulin concentration (\pm SD) in non-pregnant cows and cows during the 8th and 9th months of pregnancy

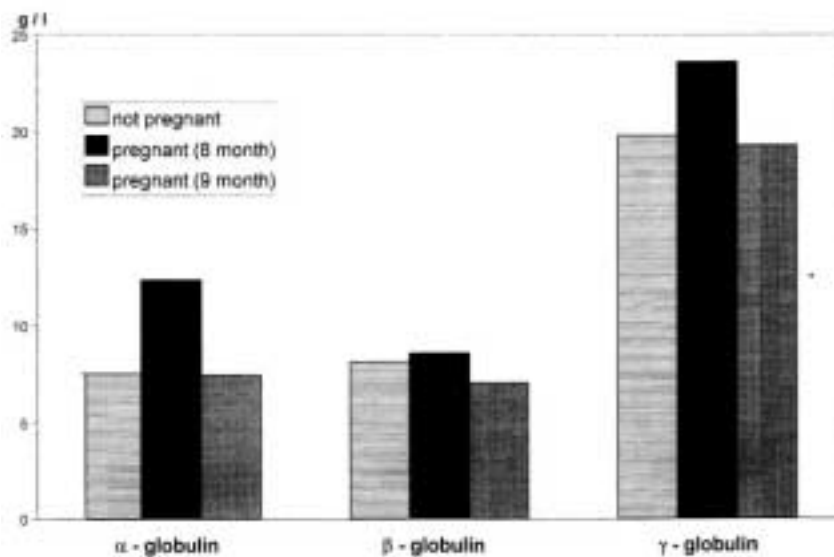


Fig. 4. Serum alpha, beta and gamma globulin concentration (\pm SD) in non-pregnant cows and cows during the 8th and 9th months of pregnancy

The results displayed a visible increase in the concentration of total serum proteins, globulin, α -globulin and β -globulin, particularly in the seventh and eighth months of pregnancy, whereas their decrease is visible in the ninth month of pregnancy (Figs 1 and 2). If mean values of total serum protein concentrations and individual protein fractions in the serum of non-gravid cows and cows in the 8th month of pregnancy are compared, it is evident that there are very significant differences in total protein, albumin, globulin, α -globulin and β -globulin concentrations, whereas the difference in the concentration of γ -globulin and A:G ratio is not significant (Table 2, Figs 3 and 4). If the results obtained in the eighth and in the ninth months of pregnancy are compared in the same way, it can be noted that in the ninth month of pregnancy the concentrations of globulin, α -globulin and β -globulin are very significantly decreased, that the total serum protein concentration is significantly decreased, and that albumin concentration is significantly increased, whereas the difference in γ -globulin recorded in the eighth and in the ninth months of pregnancy is not significant (Table 3, Figs. 3 and 4). A:G ratio very significantly decreased in the 9th month of pregnancy (Table 3).

Table 3. Concentrations of total proteins and individual protein fractions in the serum of cows in the eighth and ninth months of pregnancy (mean \pm SD)

Parameter	8 th month of pregnancy	9 th month of pregnancy
Total protein (g/l)	75.5 \pm 4.50	69.0 \pm 3.80 ^b
Albumin (g/l)	31.5 \pm 1.70	32.0 \pm 2.20 ^b
Globulin (g/l)	44.0 \pm 5.20	36.5 \pm 3.70 ^a
α -globulin (g/l)	12.34 \pm 2.57	7.61 \pm 0.81 ^a
β -globulin (g/l)	8.58 \pm 1.05	7.74 \pm 1.57 ^c
γ -globulin (g/l)	23.49 \pm 3.53	19.22 \pm 2.40 ^a
A:G ratio	0.7 \pm 0.12	0.9 \pm 0.12 ^a

^a=P<0.01 very significant; ^b=P<0.05 significant; ^c=P>0.05 non significant

Discussion

Mean values of total serum protein, albumin, globulin and γ -globulin concentrations, as well as the values of the A:G ratio, are found within the

referential values (Table 1) reported by KANEKO (1989) for the listed categories until the seventh month of pregnancy, whereas α_2 -globulin concentration is above the upper limit of the referential values (7.5-8.8 g/l) reported by KANEKO (1989), already from the sixth month of pregnancy (9.33 ± 0.93), and the α_1 -globulin concentration is somewhat lower than the referential values (8.0-11.2 g/l) reported by KANEKO (1989) in the sixth month of pregnancy (6.65 ± 1.08 g/l). In the seventh and in the eighth months of pregnancy the total serum protein (74.8 ± 3.48 g/l in the 8th; 75.7 ± 4.50 g/l in the 9th month of pregnancy), globulin (42.6 ± 5.60 g/l in the 8th; 44.0 ± 5.20 g/l in the 9th month of pregnancy), α_2 -globulin (11.56 ± 1.96 g/l in the 8th; 12.34 ± 2.57 g/l in the 9th month of pregnancy) and α_1 -globulin concentrations (24.23 ± 3.44 g/l in the 8th; 23.49 ± 3.53 g/l in the 9th month of pregnancy) are above the upper limit of referential values reported by KANEKO (1989), whereas the A:G ratio is below the lower limit of referential values, which is the result of the increase in serum globulin concentration. These observations on the influence of pregnancy on the proteinogram in cattle are in congruence with the observations of other authors (DIXON et al., 1961; LIBERG, 1977; MCGUIRE and ADAMS, 1982; KANEKO, 1989), except for the very high serum concentration of α_2 -globulin, whose continuous concentration increase during the first eight months of pregnancy in cows has not been recorded in literature. LIBERG (1977) recorded a sudden decrease of α_2 -globulin in the serum of cows immediately prior to calving, explaining this by the fact that the most frequently represented fraction of globulin in calves was the α_2 fraction, and so he was of the opinion that the decrease in concentration in the serum prior to calving in cows occurred because of the accumulation of α_2 -globulin in the mammary gland, which he additionally substantiated with the fact that immediately before calving, a component, electrophoretically corresponding to α_2 -globulins, appeared in the colostrum. If we assume that LIBERG's (1977) explanation is correct, then the α_2 -globulin concentration increase in the serum of cows in the last months of pregnancy could be the result of this, that is to say, firstly an increase of α_2 -globulin concentration would occur in the serum, as is the case with α_1 -globulins, and then α_2 -globulin would accumulate in the mammary gland, with a consequent decrease in concentration in the serum prior to calving. This assumption could be best substantiated by the electrophoresis of the colostrum and by determination of the concentration of α_2 -globulin in the colostrum. The α_2 -globulin concentration decreases between the fourth and the sixth months of pregnancy; it then suddenly increases in the seventh and in the eighth months of pregnancy, and finally, in the ninth month of pregnancy, it decreases again. LIBERG

(1977) recorded the α -globulin concentration decrease in the first three months of pregnancy, its increase until the eighth month of pregnancy, and then, again, its decrease in the last month of pregnancy. Total serum proteins, globulins and globulin fractions approach, in the ninth month of pregnancy, the values recorded in non-pregnant animals.

Conclusions

Pregnancy influences the proteinogram of blood serum in cattle. In the seventh and in the eighth months of pregnancy a significant increase in total protein, globulin, α -globulin and β -globulin occurs, whereas in the ninth month of pregnancy the values approach those recorded in non-pregnant animals.

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SAŽETAK

Istraživani su proteinogrami nesteonih krava i krava u tijeku steonosti. Zabilježena je vrlo značajna razlika u koncentraciji ukupnih proteina, albumina, globulina, α -globulina i β -globulina ($P < 0,01$) između nesteonih krava i krava u osmom mjesecu steonosti, dok razlika u koncentraciji γ -globulina te A:G omjera nije značajna ($P > 0,05$). Usporedbom rezultata zabilježenih u osmom i devetom mjesecu bređosti vidljivo je da se u devetom mjesecu steonosti vrlo značajno smanjuje koncentracija α -globulina i β -globulina ($P < 0,01$), značajno se smanjuje koncentracija ukupnih serumskih bjelančevina ($P 0,01-0,05$), vrlo značajno raste A:G omjer ($P < 0,01$) značajno raste koncentracija albumina ($P 0,01-0,05$) dok razlika koncentracije γ -globulina u osmom i devetom mjesecu steonosti nije značajna ($P > 0,05$).

Ključne riječi: proteinogrami, steonost, bređost, graviditet, elektroforeza, govedo
