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PROTEIN METABOLISM IN COWS AND THEIR CALVES FED FROM BUCKETS*

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Abstract

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The level of total protein, protein fractions, immune globulins in blood and colostral serum of 40 cows and in blood serum of their calves up to the age of 3 months were determined. No significant differences in the concentration of total proteins and their fractions in blood serum in cows ante and post partum were found. The concentration of total protein in colostral serum was found to be significantly higher ($P < 0.01$) than that in blood serum of cows.

The average value of total protein in blood serum of calves prior to the ingestion of colostrum was 41.7 g/l. The gamma globulin values in blood serum of calves reached the maximum after 24 hours upon the first ingestion of colostrum. The lowest gamma globulin values were found in calves of 3–6 weeks of age. Immune globulins correlated with gamma globulins and decreased below the standard in almost 50 % of calves 3 days old.

The absorbed gamma globulin from colostrum participated in increasing the total protein content in blood serum of calves. Besides the determination of serum gamma globulins and immune globulins the total protein determination by the refractometric method can therefore be used for the diagnosis of hypogammaglobulinemia in calves. The level of serum immune globulins should not fall below 20 units and should be measured from the 2nd to 7th day of calf age. Late and less frequent colostrum ingestion was found to be one of the causes of low immune globulin levels.

Cow, calf, blood serum, colostrum, proteins, albumins, globulins, gamma globulins, immune globulins, electrophoresis, hypogammaglobulinemia, metabolism.

Blood serum of newborn calves contains almost no gamma globulins; as early as in 1892 Ehrlich pointed out to the fact that maternal antibodies are transferred to calves by colostrum. Therefore, the calves from mothers with a syndesmochorial type of placenta are dependent on physiological and immunological condition of their dams for a certain part of the postnatal period (Přibyl 1963; Fey 1972; Logan 1977; Hajdu et al. 1977).

The normal concentration of the total serum protein of calves prior to colostrum ingestion and of foetuses is substantially lower than that found in their mothers (Baetz et al. 1971; Boguth 1953; Piskač 1961; Bárta 1962; Hojovcová 1965; Tennant et al. 1969; La Motte 1977; Bouda and Jagoš 1979). There is a good correlation between the total blood plasma protein and the immune globulins in young calves observed by Naylor et al. (1977) who estimated the colostral immunity using the total protein values in plasma. In calves 2–8 days old the total

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protein concentration in plasma was below 5.5 g/100 ml and the occurrence of intestinal disorders was found to be high. The total protein concentration in healthy calves should exceed 6 g/100 ml.

Literature data on protein fractions in the blood serum of cattle are numerous (Piskač 1961; Hojovcová 1965; Kolb 1967; Kaneko, Cornelius 1970; Vasileva 1974 and others). Recently the protein fractions of blood serum of cattle and mostly of calves were fractioned using the electrophoretic technique on acetate foils (Tennant et al. 1969; Thornton et al. 1972; Massip, Fumiere 1975; Vaissaire et al. 1976; La Motte 1977).

Gamma globulins appear in the blood of newborn calves within 60—120 minutes after the colostrum ingestion (Kaeckenbeeck et al. 1961; Aeikens 1976). The permeability time of calf intestinal mucosa is limited to 24—36 hours but with the increasing age, already 2 hours after the birth, the lowered absorption of globulins from colostrum appears (Kruse 1970, 1970a). In some cases the absorption of colostral immune globulins is quite impossible in calves 6 hours after birth (Gay et al. 1965).

Several methods have been used to determine the level of serum immune globulins in early postnatal period of calves. From the practical point of view the zinc-sulphate turbidimetric determination of immune globulins according to McEwan et al. (1970) seems to be the simplest. As to other techniques electrophoretic or radial immunodiffuse methods can be used for the gamma globulin determination (Mancini 1965).

The normal immune globulin level in calf blood serum determined by the zinc-sulphate method is more than 20 units (McEwan et al. 1970). Calves possessing this or higher level are resistant against intestinal infections and their vitality is higher. The relationship between immune globulins in calf serum and their deaths was studied by McEwan et al. (1970).

The highest immune globulin level in calf blood was achieved after 24 to 48 hours after the first colostrum was ingested. The protein composition and its quantity in colostrum is dependent on care, nutrition, age, health condition of cows and on the time period elapsing after the parturition (Logan 1977; Jagoš et al. 1977; Vasileva 1974).

The total protein in colostral serum from the first milking was measured to be in average 8.3 $\pm$ $\pm$ 3.57 g%; after 12 hours the value reached almost a half of the mentioned level (Mohar 1975). A similar value was observed by Bush et al. (1971) while the value found by Koziarowska and Koziarowski (1969) averaged 6.34 g%. Using electrophoresis on cellulose acetate usually three fractions were determined, i. e. albumins, globulin fractions and gamma globulins. Out of the total protein (8.30 g%) in colostral serum from the first milking 7.04 g% and 1.26 g% were attributed to gamma globulins and albumins, resp. Globulin intermediate fractions were determined from samples obtained upon further milking of colostrum (Mohar 1975).

Materials and Methods

The experiment was carried out on 40 high pregnant cows and their 42 calves of Czech Spotted breed in high capacity cow-houses for 600 heads. 2—5 weeks before the expected parturition and 2—4 weeks after it the clinical examination and blood sample taking was made in cows. At the same time urine was taken by catheterization and the pooled sample of colostrum from the first milking was collected.

Immediately after birth calves were transferred into individual cages in a separated calf house and at the age of 14—18 days moved into another calf house. Twice a day the calves were given colostrum from buckets and from the 6th day the feed was changed to a milk-substitute Laktosan A. Daily colostrum ration was 4—5 litres and that of milk-substitute Laktosan A 6—7 litres. The TK-1 mixture (concentrate) for calves was supplemented from the 11th day, meadow hay from three weeks of calf age. The mean weight of calves prior to colostrum ingestion was 42.4 kg. The health condition of calves was followed from birth to the age of 3 months. The blood samples were taken from v. jugularis prior to colostrum ingestion and then 1, 3, 7, 21, 42, 56 and 84 days after the colostrum ingestion.

The total protein in blood and colostral sera was determined photometrically using the Bio-test kit (biuret method) (Lachema Brno). The electrophoretic fractionation of proteins of the blood and colostral sera on acetate foils on a Microphor Boskamp instrument took 20 minutes. The evaluation of a percentage proportion of individual fractions was carried out using a Kipp and Zonen Densiscan instrument.

Immune globulins in blood serum of calves were determined turbidimetrically by a zinc-sulphate test according to McEwan et al. 1970.

The results obtained were computed on a Hewlet-Packard computer, model 9810 A. To evaluate the differences between individual samplings a variance analysis and a Duncan test were used.

Results

The average values of the total serum protein and its fractions of cows ante and post partum are given in Table 1. It follows from the results that there are non significant differences between the total protein and protein fractions in cows ante and post partum. The concentration of proteins and that of protein fractions in colostral serum from the first milking is given in Table 2. The level of the total protein in colostral serum was found to be significantly higher than that in cow blood serum.

The dynamics of concentration changes in total protein, protein fractions and immune globulins in the blood serum of calves and its dependency on age follows from Tables 3 and 4. Prior to the first colostrum ingestion the mean values of total protein in blood serum of calves were measured to be 41.7 g/l; 24 hours after the first ingestion of colostrum a significant increase of these values occurred ($P < 0.01$).

As far as the protein fractions are concerned, the most pronounced changes were noted in gamma globulins, reaching the highest value within 24—72 hours

Table 1
The mean values of blood serum protein of cows ante and post partum

Index followed	Cows ante partum	Cows post partum	*P*
Total protein g/l	76.4 ± 7.2	73.1 ± 7.0	—
Albumin g/l	42.4 ± 6.3	39.8 ± 5.3	—
Alpha globulins g/l	6.4 ± 1.9	6.6 ± 2.1	—
Beta-globulins g/l	8.3 ± 3.6	8.7 ± 1.8	—
Gamma-globulins g/l	19.3 ± 3.5	18.0 ± 3.7	—

Table 2
The mean values of proteins in colostral serum

Total protein g/l	Albumins g/l	Alpha-globulins g/l	Beta-globulins g/l	Gamma-globulins g/l
117.0 ± 33.0	14.3 ± 5.5	4.1 ± 2.8	2.5 ± 2.5	96.1 ± 5.7

Table 3
The mean values of the total protein, immune globulins and gamma-globulins in the blood serum

Animal	Age (days)	Total protein g/l		Immune globulins (units)		Gamma-globulins g/l	
		$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s
Cows	Ante partum	76.4	7.2	32.8	6.1	19.3	3.5
Calves (T)	Without colostrum	41.7	3.9	1.4	1.6	1.0	0.9
T	1	56.4	9.3	21.1	8.4	15.5	5.2
T	3	57.1	9.0	20.0	7.5	14.1	4.9
T	5—7	54.9	8.0	17.1	5.9	11.4	4.2
T	14	52.3	5.2	15.1	5.9	9.5	3.5
T	21	53.5	4.4	14.9	5.2	8.8	3.1
T	42	54.9	5.4	15.9	5.0	9.4	3.1
T	56	58.3	4.9	20.2	4.3	12.1	3.1
T	84	61.7	8.4	23.8	6.8	12.5	3.8

Table 4
The mean values of albumins, alpha-globulins, beta-globulins in the blood serum

Animal	Age (days)	Albumins g/l		Alpha-globulins g/l		Beta-globulins g/l	
		$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s
Cows	Ante partum	42.4	6.3	6.4	1.9	8.3	3.6
Calves (T)	Without colostrum	23.6	4.3	12.3	2.2	4.8	2.0
T	1	22.0	5.3	11.5	2.9	7.4	2.7
T	3	24.2	5.1	11.2	2.3	7.6	2.0
T	5–7	23.4	4.3	11.2	2.2	8.9	2.9
T	14	26.9	4.0	8.1	2.1	7.8	2.3
T	21	29.7	3.7	6.3	2.9	8.7	1.6
T	42	30.8	4.9	4.8	1.9	9.9	2.0
T	56	31.8	5.2	5.3	2.5	9.1	1.9
T	84	36.7	5.2	3.5	1.5	9.0	2.2

after the first colostrum ingestion. From day 7 to 42 of calf age the concentrations of gamma globulins in blood serum were lower, their increase, as well as that of total protein, appearing again in the 2nd and 3rd months.

Immune globulins in the calf blood serum, determined by the turbidimetric method, correlated with gamma globulins. In three-day old calves the level of immune globulins decreasing below 20 units was found in almost 50 % of animals.

Discussion

There were no significant differences in the concentration of total proteins and their fractions in blood serum of cows prior to and after parturition. The attention should be focused particularly at the colostral serum containing a significantly higher concentration of the total protein than the blood serum of cows. Also gamma globulins in colostral serum were approximately three times higher. Prior to colostrum ingestion the concentration of the total protein in calf serum does not exceed 41.7 g/l, the level of gamma globulins reaching 2.5 % out of total protein. After the ingestion of sufficient amount of colostrum a significant increase occurred not only in the case of protein levels but also in vitamins and activities of some enzymes (Bouda et al. 1979; Jagoš et al. 1979).

The highest values of gamma globulins and immune globulins were found in the blood serum of calves 24 hours after the first colostrum ingestion. Penhale et al. (1973) arrived to a similar conclusion. The immune globulin values correlated well with those of gamma globulin; essentially, in both cases gamma globulins were determined. In following days the levels of gamma globulins and immune globulins gradually decreased. The lowest values of gamma globulins, immune globulins and of total protein were found within 3rd to 6th week. Logan (1974) and Dardillat (1974) who followed antigens after the first colostrum ingestion arrived at a similar conclusion. The resistance of organism is connected with the decrease of these values; it is, therefore, a period during which the colostral immunity is lowered and the antibody production in the calf organism is still insufficient. It is, therefore, necessary to select the most suitable period for the immunization of calves since it is generally known that the immunization carried out before the disappearance of colostral antibodies is of low efficiency.

In our experiment the levels of gamma globulins determined by electrophoresis on acetate foils in the blood serum of calves after colostrum ingestion were not

lower than 8.8 g/l (16 %). The values correspond to those reported by Tennant et al. (1969) and Vaissaire et al. (1976). Approximately the same range of gamma globulin levels was obtained by Hojovcová (1965) although in this case the paper electrophoresis was employed for the separation of protein. In no case are the values of protein fractions in the blood serum of calves found by us comparable with those obtained by LaMotte (1977) who measured the concentration of gamma globulins after the colostrum intake and found it to be only 0.13 g/100 ml.

The determination of gamma globulin of immune globulin levels in the blood serum of calves is of first rate importance when the causes of high mortality and morbidity are being sought. The determination of gamma globulins and immune globulins was carried out simultaneously in order to recommend the simplest and sufficiently reliable method. It follows from the comparison of the obtained results that the turbidimetric method of determination of immune globulins is satisfactory.

Gamma globulins predominantly participate in the increase of the total protein of calves and therefore the determination of the total protein concentration in the blood serum could be of use when diagnosing hypogammaglobulinemia. Particularly the refractometric method is rapid and simple as far as instruments are concerned and was also recommended for calves and lambs by Naylor et al. (1977) and Reid and Clifford (1974), resp.

When the diagnosis of hypogammaglobulinemia using the immune globulin determination is carried out it is necessary to take blood samples from calves of certain age. According to our results the period from 24 hours to 7 days after the ingestion of colostrum seems to be the most suitable. As we have found a considerable decrease in immune globulin level on the day 14 of calf age we do not agree with the opinion of Slanina et al. (1976) who consider the age up to the 4th week as being convenient for immune globulin determination by the turbidimetric method. The immune globulin level in the blood serum of calves aged 7 days should not, according to our opinion, drop below 18—20 units and according to McEwan (1970) under 20 units. When evaluating the immune globulin and gamma globulin levels in the blood serum of calves we have found that in almost 50 % of animals examined the immune globulin levels decreased below the above mentioned value.

When studying the relationship between the immune globulin level in the blood serum and the mortality of calves McEwan et al. (1970a) found that, if the immune globulin level was 0—10 units, 30.7 % of calves died of septicemia whereas the death loss of calves was not observed if the immune globulin level exceeded 20 units. It has recently been found that at lowered immune globulin levels a respiratory syndrome was observed more frequently (Thomas and Swan 1973).

We have also found that the immune globulin level is, apart from other factors, influenced also by a timely ingestion of sufficient amount of colostrum of a good quality. The absorption of colostral gamma globulin from intestines is limited to 24—36 hours and it drops with the increasing time after parturition. If colostrum was given within three hours after birth the highest average immune globulin levels were found in the serum of calves; if the colostrum was given later than 6 hours after birth the immune globulin levels were significantly lowered (as much as 35 %) ($P < 0.01$).

The delayed colostrum ingestion was one of the main causes of low immune globulin levels in the blood serum of calves in our experiment. Out of the total

amount of calves 19.4 % were given colostrum in 3 hours after birth, 44.4 % in 3 to 6 hours but a high percentage of calves received colostrum even later (5.5 % later than 10 hours). Less frequent feeding (twice a day) is also considered to be a great inadequacy. It follows from our findings that the immune globulin level in the blood serum of calves is first of all dependent on the first ingestion of colostrum which should be effected within 3 hours, the quantity being 2 litres. Great differences were also found in the concentration of total proteins in the colostral serum of individual cows. This relationship is being further studied and the results will be published when a higher number of experiments is evaluated. It follows from our experiments that the relationship between a mother and her calf shoud be studied from all aspects. The health condition of calves is dependent on nutrition and a state of health of cows, on nutrition and care for calves, on respecting the physiological requirements of young animals, on zoohygienic conditions and on antiinfectious measures.

Metabolismus bílkovin u krav a jejich telat napájených z nádob

Byly studovány hladiny celkové bílkoviny, bílkovinných frakcí, imunoglobulinů v krevním a kolostrálním séru 40 krav a v krevním séru jejich telat do stáří 3 měsíců. V koncentraci celkových bílkovin a jejich frakcích v krevním séru u krav před a po porodu nebylo podstatných rozdílů. Koncentrace celkové bílkoviny v kolostrálním séru byla významně vyšší ($P < 0{,}01$) než v krevním séru krav.

Průměrná hodnota celkové bílkoviny v krevním séru telat před kolostrální výživou byla 41,7 g/l. Hodnoty gama-globulinů v krevním séru telat dosáhly maxima za 24 hodin po příjmu první dávky kolostra. Nejnižší hodnoty gama-globulinů byly zjištěny u telat ve stáří 3—6 týdnů. Imunoglobuliny korelovaly s gama-globuliny a u 3 denních telat byly sníženy pod normu téměř u 50 % zvířat.

Na zvýšení celkové bílkoviny v krevním séru telat se podstatně podílely absorbované gama-globuliny z kolostra. Z těchto důvodů pro diagnostiku hypogamaglobulinemií u telat kromě určení gama-globulinů nebo Ig v séru může být použito i stanovení celkové bílkoviny v séru refraktometrickou metodou. Ig v krevním séru telat by neměly klesat pod 20 j. a měly by být stanoveny od 2. do 7. dne stáří telat. Jednou z hlavních příčin nízkých hladin Ig u telat bylo pozdní a málo časté podávání kolostra.

Метаболизм белков коров и их телят, поенных из сосудов

Проводились изучения уровней общего белка, белковых фракций, иммуноглобулинов в кровяной и молозивной сыворотке 40 коров и в кровяной сыворотке их телят в возрасте до 3 месяцев. В концентрации общих белков и их фракций в кровяной сыворотке коров до и после отела не наблюдалось существенной разницы. Концентрация общего белка в молозивной сыворотке была значимо выше ($P < 0{,}01$) по сравнению с кровяной сывороткой коров.

Среднаяя величина общего белка в кровяной сыворотке телят перед молозивным питанием достигала 41,7 г/л. Максимальной концентрации достиг общий белок (57,19 г/л) у трехдневных телят, последующие дни имело место постепенное понижение.

В процентном отношении фракций белков в кровяной сыворотке телят самые большие изменения имели место в фракции гамма-глобулинов и альбу-

минов. Гамма-глобулины в кровяной сыворотке телят до молозивного питания достигали 2,5 % общего белка, максимума достигли в течение суток после приема первой дозы молозива и с постепенным возрастом наблюдалось их понижение с минимальными величинами у телят в возрасте 3—6 недель. Иммуноглобулины находились в корреляции с гамма-глобулинами и в случае телят в возрасте 3 дней они почти у 50 % животных находились ниже нормы. Подобным образом обстояло дело с гамма-глобулинами.

В повышении общего белка в кровяной сыворотке телят принимали существенное участие поглощенные из молозива гамма-глобулины. По этой причине, помимо определения гамма-глобулинов и иммуноглобулинов в сыворотке, для диагностики гипогамма-глобулинемий у телят можно прибегнуть также к опделелению общего белка в сыворотке рефрактометрическим методом. Иммуноглобулины в кровяной сыворотке телят не должны доходить ниже 20 ед. их следует определить на 2—7 день возраста телят. Одна из причин низкого уровня иммуноглобулинов телят заключется в поздней и не очень частой подаче молозива. Из нашего исследования вытекает, что на состояние здоровья телят оказывает влияние ряд факторов, первоочередным из числа которых является питание и состояние здоровья коров, комплексные санитарные и противоэпидемические меры, хороший уход и последовательное соблюдение физиологических требований молозивников.

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