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EFFECT OF MILK REPLACER ON CALF REARING PERFORMANCE AND HEMATOLOGICAL AND BIOCHEMICAL BLOOD INDICES

Mariusz Bogucki[1], Monika Bogusławska-Tryk[2], Magdalena Stanek[2], Iwona Kuropatwińska[3], Jozef Bujko[4]

[1]Department of Animal and Nutrition Breeding, Bydgoszcz University of Science and Technology, Mazowiecka 28, 85-084 Bydgoszcz, Poland
[2]Department of Animal Physiology and Physiotherapy, Bydgoszcz University of Science and Technology, Mazowiecka 28, 85-084 Bydgoszcz, Poland
[3]Rolhod Sp. z o.o., Wąsewo 16, 88-230 Piotrków Kujawski, Poland
[4]Institute of Nutrition and Genomics, Faculty of Agrobiology and Food Resources, Slovak University of Agriculture in Nitra, Slovakia

ABSTRACT

The aim of this study was to evaluate the effect of the type of milk replacer on the production results of calf rearing and hematological and biochemical parameters of their blood. Calves (heifers) of the Polish Holstein-Friesian breed, divided into two groups of 18 animals each, were maintained and fed equally, except for milk replacers used from the 31st to the 90th day of calf life. Group A received milk replacer based on skimmed milk powder, and group B received milk replacer based on whey powder. Production parameters were calculated and hematological and biochemical blood indices were determined. Better calf rearing results were found when the formula A was used. Calves, despite a lower initial weight at 30 days of age, reached a higher final weight at 90 days of rearing. There was a significantly higher number of leukocytes and erythrocytes in the blood of gorup A clves, whereas in group B there was a higher average red blood cell volume and average weight of hemoglobin in a red blood cell. In addition, there were significant differences in the levels of total cholesterol and HDL cholesterol in the blood of calves fed different milk replacers ($P \leq 0.05$).

Key words: calves, nutrition, milk replacer, hematology, blood biochemistry

INTRODUCTION

The proper growth, development and health status of calves depend on a combination of factors, both genetic and environmental [Quigley et al. 2006, Klinkon and Ježek 2012, Khan et al. 2018, Teneva et al. 2020]. The cost of rearing is very high and forces breeders to look for cost savings, which, however, should not inhibit the animal's growth rate. Calves and juveniles must grow at a rate fast enough, so that it would be possible to mate a heifer at 13–14 months of age, and a trouble-free birth would take place at about 22–23 months of age, with a body weight of more than 560 kg. In terms of the potential performance of the cow, saving on calf rearing is unjustified [Blum 2006, Kowalski 2015].

According to Kowalski [2015], the results of some studies [Brown et al. 2005, Soberon et al. 2012, Soberon and Van Amburgh 2013] indicate that by providing calves during the rearing period with a rapid growth rate averaging more than 650 g per day in the first 2 months of life, a higher milk yield can be 'programmed' in the future. Such rapid growth, resulting from proper nutrition during the first 60 days of life, enables optimal growth of the glandular tissue of the udder, ensures strengthening of the immune system, stimulates proper development of all systems, including the skeleton, circulatory (heart) and digestive (especially liver) systems. Moreover, besides a higher productivity, such well-balanced nutrition can increase the longevity of the cow and allow her to fully realize her genetic potential. According to Soberon et al.

bogucki@pbs.edu.pl

[2012], a weight gain of 1,000 g per day results in an increase of 850 kg in the first lactation and almost 890 kg of milk in the second. According to the aforementioned authors, the effect of growth rate and nutrient intake of the calf during the rearing period has a greater impact on milk yield than the genetic potential of the cow.

An important factor affecting animal health is the quality of the feed ration. It is particularly important during the early development period, when nutrient requirements are high and liquid feeds are the basis of nutrition. An optimally balanced diet, with proper environmental conditions, guarantees good animal condition, and this translates into satisfactory production results. There are many milk replacers available on the market, and the effectiveness of their use is mainly influenced by the chemical and component composition of the formulation [Bogucki 2022]. They can affect the body in different ways, causing a number of either positive or negative changes in the system.

The effect of the type of milk replacer on the health of calves can be characterized by clinical blood indicators, such as white and red blood cell count, hematocrit index value, hemoglobin content and red blood cell parameters (MCV, MCH and MCHC) [Kelada et al. 2012]. The results of blood biochemical component analyses are also a reliable source for animal health assessment [Lepczyński et al. 2011]. They show the influence of various environmental factors on the homeostasis [Florek et al. 2009]. The levels of blood biochemical parameters related to nitrogen metabolism (TP, ALB, GLOB and UREA), fat metabolism (TG, TC, HDL and LDL), as well as the indices of the liver function (ALT, AST and BIL) inform about the normal functioning of the organism.

The aim of this study was to evaluate the effect of the type of milk replacer used on calf rearing performance and hematological and biochemical blood parameters.

MATERIAL AND METHODS

The study was conducted in a dairy cattle herd of 500 animals (including 300 cows). The experiment involved 36 calves (heifers) of the Polish Holstein-Friesian breed, which were divided into two groups of 18 animals each (same age – 30 days, similar birth weight +/– 3 kg). During the experiment, both groups were maintained under the same conditions (until 30 days of age in individual pens, from 31 to 90 days of age in group pens (6 calves each), fed according to the same program, the same feeds, except milk replacer (MR), used from 31 to 90 days of age of calves. Group A received a milk replacer based on skimmed milk powder. Group B, on the other hand, received a second milk replacer based on whey powder.

Feeding scheme for calves from 1 to 90 days of age:

- until day 5: colostrum (IgG>100 $g \cdot l^{-1}$),
- from day 6 to 30: whole milk 2 x 4 l, muesli-type starter at will,
- from day 31 to 60: milk replacer 2 x 4 l (dosed according to the manufacturer's recommendations), muesli-type starter at will, additionally from day 45 concentrate mix at will,
- from 61 to 90 days: milk replacer 2 x 4 l (from 80 to 85 days 1 x 4 l, from 86 to 90 days 1 x 2 l), muesli-type starter at will, concentrate mix at will, hay at will, corn silage at will.

After a period of rearing on whole milk (with the participation of a muesli starter in the ration), calves were weighed on day 30 and blood was drawn from them (from the zygomatic vein) by a veterinarian for hematological and biochemical determinations. Weighing of calves (to determine the final body weight in the experiment), along with blood collection, was repeated on their 90th day of life.

Based on the unit body weights of calves at the beginning and end of the experiment, the following production parameters were calculated in both groups: average initial body weight (kg); average final body weight (kg); absolute gain (kg) – the difference between the body weight of calves at the end and at the beginning of the milk replacer feeding period; daily gain (g) – absolute gain/number of days of milk replacer use in calf feeding (60 days); relative gain (%) – expressing the growth rate as a percentage ratio of absolute gain to average body weight during the study period.

Table 1. Comparison of the composition of milk replacers used in the experiment

Composition	MR	
	A	B
Skimmed milk powder	+	–
Dried whey	+	+
Refined vegetable fat	+	+
Whey protein powder	+	+
Hydrolyzed wheat protein	+	+
Dextrose, grape sugar	+	–
Gelatinized wheat starch	+	+
Soy protein concentrate	–	+
Yeast extract	+	–
Fructooligosaccharides	+	–
Chicory inulin	+	–
Egg powder	+	–
Grape seed extract	+	–

Two tubes of blood were collected from each calf each time. One standardized with anticoagulant for the determination of hematological indices, the other 10 ml for the determination of biochemical blood indices. Immediately after collection, the blood tubes were transported under refrigerated conditions to the laboratory of the Department of Animal and Nutrition Breeding, Bydgoszcz University of Science and Technology.

Table 2. Analytical composition of MRs used in the feeding of group A and B calves

Analytical components	MR, %	
	A	B
Crude protein	22.0	18.0
Crude fat	20.0	16.5
Crude fiber	0.02	0.2
Crude ash	7.7	8.5
Lysine	2.0	1.7
Calcium	0.9	0.9
Phosphorus	0.7	0.7
Sodium	0.6	0.4

The following hematological indices were determined in whole blood samples: white blood cell (WBC) count, red blood cell (RBC) count, hematocrit index (HT) and hemoglobin (HGB) content. Morphotic indices were determined using the classical chamber method (Bürker chamber). The size of the hematocrit index was determined by the microhematocrit method, while the HGB content was determined by the Drabkin method, using an Epoll 20 photometer and ready-made reagent kits. The following red blood cell indices were calculated from the results: mean red blood cell volume (MCV), mean red blood cell hemoglobin mass (MCH) and mean red blood cell hemoglobin concentration (MCHC).

Table 3. Analytical composition of starter and concentrate mixes used in the feeding of group A and B calves

Analytical components	Feed, %	
	Muesli starter	Concentrate mix
Crude protein	19.5	18.0
Crude fat	2.3	2.7
Crude fiber	4.6	6.4
Crude ash	6.6	6.6
Calcium	1.1	1.0
Phosphorus	0.5	0.6
Sodium	0.3	0.3

Blood samples for evaluation of biochemical indices were placed in sterile glass tubes without anticoagulant and centrifuged to separate the clot from the serum. Serum was determined using a MINDRAY BS-120 biochemical analyzer and reference reagents (STAMAR®, Dąbrowa Górnicza, Poland): protein levels (total protein TP), albumin (ALB), globulin (GLOB), triglycerides (TG), total cholesterol (TCH), high-density lipoprotein (HDL), low-density lipoprotein (LDL), aspartate aminotransferase (AST), alanine aminotransferase (ALT), glucose (GLC), urea (UREA) and bilirubin (BIL).

In both study groups, mean values and standard deviations were calculated for production traits, hematological blood parameters and biochemical blood indicators. One-way analysis of variance was used for this purpose. Differences between mean values were verified using the Tukey test [Statistica 2020].

RESULTS AND DISCUSSION

In our study, milk replacers from two market-leading companies were tested. More favorable calf rearing results were found when formula A was used. Calves, despite a lower initial weight at 30 days of age of 52.8 kg compared to 54.3 kg in group B, reached a higher final weight at 90 days of rearing – 118.9 kg, compared to 117.5 kg in group B. However, the statistically confirmed differences ($P \leq 0.05$) are mainly in terms of gains – absolute and daily (Table 5). Calves in group A at the time of milk replacer weaning gained 66.1 kg, while calves in group B gained 63.2 kg. Average daily gains, in turn, were at the levels of: 1101 and 1053 g. These were higher than those obtained by Hill et al. [2010]. The authors, depending on the feeding program used (milk replacers with different percentages of protein and fat), found daily calf weight gains of 650–780 g during the 86-day rearing period.

Despite ongoing improvements in manufacturing processes, milk replacers are still only substitutes for natural whole milk from healthy cows, and their use in calf rearing is a constant compromise. Growers use formulas because they are cheaper than milk, although they are aware that they still do not match the genuine. The positive thing is that the difference between the biological value of milk replacers and milk is steadily decreasing.

According to Niwińska [2017], the results of the study confirm the relationship between the increase in daily weight gains of heifers and their subsequent productivity. The so-called economic weight index of daily weight gains in the overall economic effect of the obtained milk production in Holstein-Friesian cows was also estimated. The financial impact was found to be 18% dependent on daily weight gains in the pre-weaning period [Wolfová et al. 2007].

The percentage ratio of absolute gain to average body weight during the study period, which expresses the rate of calf growth (relative gain), shows that a statistically significantly higher value ($P \leq 0.05$) characterized the

Table 4. Effect of MR type on final weight of reared calves

MR group	N	Initial weight, kg	Final weight, kg
A	18	52.8 ±2.6	118.9 ±2.9
B	18	54.3 ±2.8	117.5 ±3.1

Table 5. Effect of PMZ type on absolute, daily and relative gain of reared calves

MR group	N	Total gain, kg	Daily gain, g	Relative gain, %
A	18	66.1^{a} ±2.8	1101^{a} ±61	77.0^{a} ±4.0
B	18	63.2^{b} ±3.2	1053^{b} ±56	73.5^{b} ±4.7

$^{a, b}$ $P \leq 0.05$.

Table 6. Selected hematological blood parameters of calves fed different milk replacers

Parameter	Milk Replacer			
	A		B	
WBC, $G \cdot l^{-1}$	10.89	$\pm 2.24^{a}$	8.13	$\pm 1.68^{b}$
RBC, $T \cdot l^{-1}$	8.67	$\pm 1.07^{a}$	7.33	$\pm 1.64^{b}$
HT, $l \cdot l^{-1}$	0.36	±0.03	0.36	±0.02
HGB, $mmol \cdot l^{-1}$	7.55	±1.39	7.27	±0.55
MCV, fl	42.11	$\pm 5.55^{a}$	51.07	$\pm 10.65^{b}$
MCH, fmol	0.88	$\pm 0.18^{a}$	1.07	$\pm 0.24^{b}$
MCHC, $mmol \cdot l^{-1}$	20.86	±2.66	20.29	±1.27

$^{a, b}$ $P \leq 0.05$.

Table 7. Effect of milk replacers (MR) on biochemical indices of calf blood

Index	MR A		MR B	
Glucose	3.39	± 0.99	3.15	± 0.54
Protein	60.19	± 3.44	57.49	± 5.48
Albumin	30.76	± 1.30	30.16	± 0.87
Globulin	29.43	± 3.84	27.33	± 5.61
Urea	3.99	± 1.44	4.36	± 1.09
Cholesterol	2.31	$\pm 0.39^{a}$	1.89	$\pm 0.49^{b}$
HDL	1.58	$\pm 1.19^{a}$	1.75	$\pm 0.23^{b}$
LDL	0.32	± 0.07	0.29	± 0.08
TG	0.17	± 0.04	0.18	± 0.06
Bilirubin	3.48	± 0.89	3.66	± 0.96
ALT	21.67	± 5.85	19.89	± 4.70
AST	78.40	± 7.43	76.22	± 14.40

$^{a, b}$ $P \leq 0.05$.

group of calves fed with formula A – 77.0%, against 73.5% in the group fed with formula B (Table 5).

Table 6 shows the values of selected hematological blood parameters of calves fed various milk replacers. The results obtained were mostly within the range of reference values [Mordak 2008]. In the group fed with formula A, the number of leukocytes (WBC) and erythrocytes (RBC) in the peripheral blood of calves was found to be significantly higher ($P \leq 0.05$) compared to calves receiving formula B-based feed. However, there was no effect of the milk replacer used on the size of the hematocrit index (HT) and hemoglobin (HGB) content in the animals' blood. Analyzing the calculated red blood cell indices, there was a significantly higher mean red blood cell volume (MCV) ($P \leq 0.05$) and mean red blood cell hemoglobin mass (MCH) ($P \leq 0.05$) in the calves demilked with formula B, compared to group A. Regarding the average hemoglobin concentration in the red blood cell (MCHC), there were no significant differences between the study groups.

White blood cell (WBC) counts are important indicators for assessing immunity. Higher leukocyte levels in calves receiving a milk replacer based on skimmed milk powder could be due to the stimulation of leukopoiesis and the immunostimulating effect of the food [Low et al. 2001, Ballou 2012, Ballou et al. 2015]. Milk proteins (both α-, β- and κ-casein fractions and whey proteins) stimulate lymphocyte proliferation [Low et al. 2001, Ambroziak and Cichosz 2014]. It should be noted, however, that WBC levels did not exceed reference values [Mordak 2008] and were similar to values obtained by Mohri et al. [2007] for 84-day-old calves. It is well known that the white blood cell count in cattle does not have a high diagnostic value, and even during acute inflammation, elevations are rarely noted [Winnicka 2015]. In addition, in calves the total leukocyte count is not a constant value and fluctuates significantly, especially under severe stressors. Therefore, the WBC values obtained in the experiment may indicate the good condition and health status of the tested animals.

Reference values for individual blood parameters should take into account the age of the animals. Hematological indicators of cattle, such as the number of red blood cells, erythrocyte volume and their biochemical composition undergo significant fluctuations during the first months of life [Egli and Blum 1998, Knowles et al. 2000, Mohri et al. 2007, Benesi et al. 2012]. These changes are primarily related to the exchange of fetal hemoglobin for adult animal HGB, a decrease in erythrocyte volume, and changes in sodium and potassium concentrations in red blood cells [Brun-Hansen et al. 2006, Mohri et al. 2007, Golbeck et al. 2018, Golbeck et al. 2019]. Since the available literature lacks detailed data on erythrocyte count, hematocrit size or hemoglobin content in 12-week-old calves, therefore the results obtained were related to average reference values for this technological group [Mordak 2008]. The red blood cell (RBC) count recorded in the group of heifers fed with formula B was within the range of physiological norms, while in group A it slightly exceeded the upper limit of reference values. This may have been due to an attempt to compensate for the lower average erythrocyte volume and lower average hemoglobin mass in the red blood cell. Similarly, in the study by Brun-Hansen et al. [2006], the lower MCV was compensated for by a higher RBC count to maintain normal peripheral blood hemoglobin levels. According to Golbeck et al. [2019] the decrease in erythrocyte volume in calves is a physiological change that occurs during the first months of life and is not associated with anemia in healthy animals.

Analyzing the results obtained, it can be concluded that calves of both A and B groups were characterized by good condition and health, which translated into satisfactory rearing results.

The results of the analyses of biochemical parameters determined in the calves' blood serum are summarized in Table 7, and all their values were within the reference values reported in the literature [Winnicka 2004, Lubojemska and Kinal 2007, Radostits et al. 2009, Klinkon and Ježek 2012].

Our study showed statistically significant differences ($P < 0.05$) in the levels of total cholesterol and HDL cholesterol in blood taken at 90 days of age from calves fed different preparations. For the other analyzed biochemical parameters, there were no statistically significant differences between the groups. Since the level of biochemical indices in the blood serum indicates the general state of the organism and the changes occurring in it under the influence of stable internal and external factors, the lack of differences between the experimental groups may indicate the maintenance of homeostasis and metabolic balance in calves fed different milk replacers.

The levels of total protein, globulins and albumin were lower in the blood of calves fed formula B, but in both groups the levels of these indicators were within reference ranges [Winnicka 2004]. The concentration of total protein in blood plasma and the content of its fractions, in addition to the quantity and quality of feed, are influenced by the function of individual organs of the body, such as the permeability of the intestinal barrier and the function of the kidneys [Ciechanowicz et al. 2010]. Globulins are responsible for transporting compounds such as Ca, P, vitamins and hormones, as well as maintaining the osmotic pressure of the blood at appropriate levels [Lubojemska and Kinal 2007]. A study by Quigley et al. [2006], which analyzed the effects of the addition of various milk replacers on calf health, growth and selected blood metabolites, showed no significant differences in serum protein concentrations. Similarly, our own study showed no significant differences in the protein profile between groups of calves fed with formulas whose protein content ranged from 18% (formula B) to 22% (formula A). These results correspond with those obtained by Li et al. [2008] in calves at 60 days of age fed milk replacers containing 18 to 26% protein. Shukla [2012], studying the effect of milk replacer feeding on hybrid Holstein-Kanker calves, observed no significant difference in total protein and albumin concentrations in the serum of calves receiving different formulas. Lee et al. [2008] showed that feeding Holstein-Friesian calves high-protein and low-energy and low-protein and high-energy milk replacers did not significantly affect total protein and serum albumin concentrations at 49 days of age. Similarly, Huang et al. [2015], who analyzed the effects of various plant proteins (soy, wheat, peanut and rice) present in milk replacers on blood biochemical indices, showed that there were no significant differences in TP, ALB and GLOB levels between groups at 63 days of age in calves.

Blood glucose levels are unstable and susceptible to stress factors and increase after feeding. Studies have shown that blood glucose levels increase in animals fed a diet containing fermentable carbohydrates [Mantri et al. 2019]. Our study showed that there were no significant differences in blood glucose levels between the groups analyzed. Lee et al. [2008] observed that feeding calves high-protein and low-energy milk replacer and low-protein and high-energy milk replacer with 10% lactose did not significantly affect blood glucose levels. Similar results were obtained by Quigley et al. [2006], who confirmed that feeding calves milk replacers containing 49.16 g and 50.86 g of lactose increased plasma glucose concentrations, but slightly.

Serum urea concentration reflects the conditions of protein metabolism and amino acid balance in the animals' diet and is also a picture of rumen and liver function [Quigley et al. 2006, Huang et al. 2015]. A lower urea concentration is when the amount of protein in the ration is lower. Our own research showed that there were no significant differences in urea concentration between the groups, despite differences in the protein content of the formulations (18% and 22%). In addition, urea concentrations were within reference values, suggesting normal liver and kidney function in calves in both study groups. Lee et al. [2008] showed significant differences in blood urea concentrations in calves at 49 days of age fed high-protein (25% protein) and low-protein milk replacer (21% protein). Similar differences were observed by Quigley et al. [2006] on day 56 of calves fed milk replacers containing 20 to 28% protein, and Li et al. [2008] at day 60 of calves fed formulas containing 18 to 26% protein.

Changes in the values of blood lipid indices, i.e. TC and HDL and LDL, are important indicators that assess metabolic processes [Florek et al. 2009]. Lee et al. [2008] showed higher concentrations of total TCH cholesterol in a group of calves fed high-fat milk replacer, which could be attributed to increased fat supply and absorption. This was also confirmed by our own research, which showed significantly lower TCH concentrations in group B, a group of calves fed formula B, which contained less crude fat (16.5%) than formula A (20%). According to the literature, for proper functioning of the body, the concentration of HDL levels should be above 40% of TC [Florek et al. 2009, Klebaniuk and Rocki 2011]. In the experiment conducted, the percentage of HDL ranged from 68 to 92%. TGs are the main form of fat storage in the body, being accumulated in fat cells and released into the bloodstream when needed, however, the study showed no differences in TG levels between groups.

AST is present in various tissues and is a sensitive indicator of soft tissue damage. The activity of this enzyme decreases after the first week of calves' life, and slowly increases from 42 to 84 days of age [Klinkon and Ježek 2012]. In our study, serum total bilirubin levels were within the reference values for adult animals, which may be related to improved glomerular filtration and liver function with age. The study in question showed that AST, ALT and BIL levels were not significantly different between the groups fed formula A and B.

CONCLUSIONS

More beneficial results of calf rearing were found with the use of preparation A (based on skimmed milk powder). Calves, despite a lower initial weight at 30 days of age, amounting to 52.8 kg against 54.3 kg in group B, reached a higher final weight at 90 days of rearing, 118.9 kg, against 117.5 kg in group B. The group fed with formula A had a significantly higher number of leukocytes and erythrocytes in the blood, while group B had a higher average red blood cell volume and average weight of hemoglobin in the red blood cell. Our own research showed significant differences in the levels of total cholesterol and HDL cholesterol in blood taken at 90 days of age of calves fed different formulas. However, the milk replacers used in most of the hematological and biochemical blood indicators analyzed were within the reference values, indicating undisturbed metabolism and homeostasis of the body. This confirms that the formulas used can be used in the feeding of calves without negative effects on their health.

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WPŁYW RODZAJU PREPARATU MLEKOZASTĘPCZEGO NA WYNIKI ODCHOWU CIELĄT ORAZ WSKAŹNIKI HEMATOLOGICZNE I BIOCHEMICZNE KRWI

STRESZCZENIE

Celem pracy była ocena wpływu rodzaju preparatu mlekozastępczego na wyniki produkcyjne odchowu cieląt oraz parametry hematologiczne i biochemiczne ich krwi. Cielęta (jałówki) rasy polskiej holsztyńsko-fryzyjskiej podzielone na dwie grupy po 18 zwierząt, utrzymywano i żywiono jednakowo, z wyjątkiem preparatów mlekozastępczych stosowanych od 31 do 90 dnia życia cieląt. Grupa A otrzymywała preparat mlekozastępczy na bazie odtłuszczonego mleka w proszku, a grupa B preparat mlekozastępczy na bazie serwatki w proszku. Obliczono parametry produkcyjne oraz oznaczono wskaźniki hematologiczne i biochemiczne krwi. Stwierdzono korzystniejsze wyniki odchowu cieląt w przypadku stosowania preparatu A. Cielęta, pomimo niższej masy początkowej w 30 dniu życia, w 90 dniu odchowu osiągnęły wyższą masę końcową. W grupie cieląt odpajanych preparatem A stwierdzono istotnie wyższą liczbę leukocytów i erytrocytów we krwi, natomiast w grupie B wyższą średnią objętość krwinki czerwonej oraz średnią masę hemoglobiny w krwince czerwonej. Ponadto wykazano istotne różnice w poziomie cholesterolu całkowitego i HDL w krwi cieląt żywionych różnymi preparatami mlekozastępczymi ($P \leq 0.05$).

Słowa kluczowe: cielęta, żywienie, preparat mlekozastępczy, hematologia, biochemia krwi

Mariusz Bogucki https://orcid.org/0000-0002-7524-7417
Monika Bogusławska-Tryk https://orcid.org/0000-0001-7481-1701
Magdalena Stanek https://orcid.org/0000-0003-4065-6154
Jozef Bujko https://orcid.org/0000-0003-4864-0384