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Original article

CLINICAL AND BIOCHEMICAL ASSESSMENT OF A PROBIOTIC FEED SUPPLEMENT APPLICATION ON CALVES

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Summary

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The intensification of animal husbandry prompts producers to use modern technologies that are safe for humans and promote the welfare and health of animals. Despite the long time spent studying the effectiveness of probiotics, questions regarding the optimal composition and period of rational use of probiotic feed additives (PFA) for young animals, in particular as alternative to the use of antibiotics remain relevant. The purpose of the study was to evaluate the effect of a probiotic feed additive on clinical and biochemical parameters in healthy calves (Black-and-white breed, 1 day to 1 year of age) from different physiological groups and preventive efficiency. The use of PFA (*Bacillus subtilis* and *Bacillus licheniformis*) at all stages of growing calves had a positive effect on growth indicators, increased live weight of animals and average daily weight gain, allowing for more efficient use of feed, mitigation of the production stress and obtaining greater profits from intensive animal breeding, sales of ecological products, and the reduction of costs for veterinary services. Thus, it is advised to feed PFA in the neonatal period for the formation of intestinal microbiocenosis; at the age of 1–2 months: in order to correct the immune reactivity; at the age of 3 months – to improve the processes of scar formation. The PFA, additionally supplemented with *Sacharomyces serevisiae*, can correct the immune reactivity of 1–2 months old calves; improved the processes of scar formation at the age of 3 months; and prepared heifers for mating at the age of 12 months. The obtained results experimentally substantiated the use of probiotics within the framework of the WHO Global Action Plan on Antimicrobial Resistance – it has been proven that the prevention of diarrhoea and/or bronchopneumonia as a result of the use of probiotics, even in one calf, allowed avoiding at least 4–6 days of antibacterial therapy that reduced the risks of formation of antibiotic-resistant bacterial clones, in particular zoonotic agents.

Key words: bronchopneumonia, calves, diarrhoea, probiotic feed additive

INTRODUCTION

Bacillus spp. cultures as well as *Sacharomyces cerevisiae* are used in feed additives to improve feed digestibility and prevent disease in industrial cattle husbandry systems in order to minimise the use of antibiotics (Lytvyn & Polishchuk, 1990; Xiao *et al.*, 2016; Gibson *et al.*, 2017), which definitely affects the preservation and productivity of animals. Feeding yeasts stimulates and increases the number of anaerobic bacteria because the respiratory activity of yeasts protects the anaerobic bacteria of the rumen from oxygen-induced damage (Newbold *et al.*, 2007).

Saccharomyces and BPS-44 (*Bacillus subtilis*) preparations increased the intensity of post-vaccination immunity against Gumboro disease and antioxidant potential in broiler chickens (Romanovych *et al.*, 2019). Other authors (El-Ghany *et al.*, 2022) have shown the effectiveness of the use of postbiotics and probiotics in the schemes of combined therapy of experimental necrotic enteritis of broiler chickens, caused by *Cl. perfringens.*

A number of scientific publications confirmed the effectiveness of yeast feed additives (Desnoyers *et al.*, 2007; Ermakova *et al.*, 2021). However, Bouchicha *et al.* (2022) found no positive effect from the use of probiotics (*Saccharomyces cervicae, Lactobacillus spp*.) for prevention of the development of ketosis in goats in the terminal stage of pregnancy.

Other authors (Ermakova *et al.*, 2021) affirmed that the use of *Bacillus subtilis* DSM 32424 probiotic culture with drinking water by laying quails inhibited the proliferation of faecal staphylococci and contributed to increased yolk carotenoids content. They suggested that the inclusion of this probiotic in the diet of productive quails can have beneficial results for both laying hens and their offspring. Lamari *et al.* (2021) have published data regarding the effectiveness of the use of a probiotic feed additive in mastitis in cows. Other researchers (Ban & Guan, 2021) deemed necessary to study the effect of probiotics in more details by selecting the optimum composition of the microflora depending on the age of the animals. This is especially true for calves because the animal microbiome must be able to change its diet from digesting milk to roughage in a fairly short period of time. The microflora of the alimentary tract (especially the forestomachs) changes over time. The neonatal period is the most risky for digestive diseases in ruminants; during the first month of calf life, a microbial population similar to that of adult cows is formed (Mishurnova, 1993), that is, the composition of probiotic cultures should be chosen in a way to ensure the highest economic efficiency of each period of raising productive animals.

Our experiments were based on critical and physiologically important age-related risk factors under the conditions of keeping young cattle. Therefore, taking into account the economic component and physiological features, our attention was focused on the regulations for the use of PFA in young cattle.

The aim of the present experimental research was to evaluate the effectiveness of a probiotic feed additive (PFA) in calves of different ages under production conditions according to the dynamics of clinical and biochemical indicators.

MATERIALS AND METHODS

This study was performed at the Department of Epizoology, Microbiology and Virology of the National University of

Life and Environmental Sciences of Ukraine during 2019–2020. Research was conducted in accordance with the requirements of the Ukraine Law on Protection of Animals from Cruelty, and Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes.

The research was conducted in the farms of the Kyiv and Chernihiv regions of Ukraine on 180 calves of the Black and white breed; 11 experimental and 7 control groups of calves of different ages (10 animals in each; n=180) were formed. PFA of different composition and different pharmaceutical forms were used (Table 1). PFA contained cultures of *Bacillus subtilis* 12P-130 and *Bacillus licheniformis,* 6×10^9 CFU/g/mL in two pharmaceutical forms: liquid (L1) – for drinking with milk, and dry (D1) – for feeding with compound feed. Comparison drug contained additionally a *Saccharomyces cerevisiae* yeast culture, 1×10^9 CFU/g/mL also in two pharmaceutical forms: liquid (L2) and dry (D2).

Experimental design

Experimental studies were conducted in seven stages:

- Stage I: animals (newborn calves) received PFA D1 with milk at a dose of 5 g and 10 g per animal/day for 10 days (experimental 1; experimental 2; control; n=10);
- Stage II: animals (14-day-old calves) received PFA L1 and PFA L2 at a dose of 10 mL/day for 12 days (experimental 1; experimental 2; control; n=10);
- Stage III: one-month-old calves (n=20) received PFA D1 at a dose of 5 g for the first 20 days and at a dose of 10 g per animal/day for the next 40 days (experimental 1; control 1; n=10);
- Stage IV: animals (calves aged 2 and 3 months) received PFA L2 at a dose of 10 mL (5 mL twice) per animal/day for 3 weeks (experimental 1; experimental 2; control 1; control 2; n=10);
- Stage V: animals (4-month-old calves) received PFA D1 and D2 with milk at a dose of 10 g per animal/day for 2 weeks (experimental 3; experimental 4; control; n=10);
- Stage VI: animals (12-month-old heifers) received PFA D2 with feed at a dose of 40 g and 20 g per animal/day for 3 weeks (experimental 1; experimental 2; control; n=10).

In order to determine the effect of the studied PFA samples on biochemical parameters – enzymatic activity of AST, ALT, and GGT; calcium, phosphorus, glucose, total proteins, albumins, urea and serum bactericidal activity (SBA), blood samples were collected from the animals before and after application of PFA vari-

Table 1. Characteristics of probiotic feed application (PFA) variants used in studies

Drug	Pharmaceutical form	Code	PFA composition
1	Dry, D	D1	*Bacillus subtilis* 12P-130; 5×10^9 CFU/g/mL *Bacillus licheniformis* 12P-896; 5×10^9 CFU/g/mL
	Liquid, L	L1	
2	Dry, D	D2	*Bacillus subtilis* 12P-130; 1×10^{10} CFU/g/mL *Bacillus licheniformis* 12P-896; 1×10^{10} CFU/g/mL *Saccharomyces cerevisiae* AF 338 ; 1×10^9 CFU/g/mL

ants. The serum biochemical indicators were assayed using an automatic biochemical analyzer LabLine-010 (Austria) with commercial kits (CORMAY, Poland). When determining the SBA, the *Escherichia coli* 2017/4 (*stx2*) strain from the NUBIP collection was used as a test microorganism (Vlizlo *et al.*, 2012).

The results were subjected to one-way analysis of variance (ANOVA) and Dunnett's test at a level of significance $P<0.05$ using GraphPad Prism 8.3.0 for Windows.

RESULTS

The epizootic examination showed that calf diseases such as *Escherichia coli* infection, oral and coronavirus infections, and infectious rhinotracheitis were registered in the farm, but without signs of epizootic. Calves suffering from diarrhoea and/or respiratory tract disorders were treated according to the traditional scheme used at the farm with empirical use of antibiotics.

Stage I – newborn calves (n=30)

PFA D1 was given immediately after birth at a doses of 5 g and 10 g per animal/day with milk. In the calves of the first experimental group, the average daily live weight gain (LWG) increased by 15.0%, and in the second experimental group by 14.5% ($P<0.002$) compared to the control group (Table 2).

A significant increase in the level of SBA was recorded in the animals from the experimental groups after the cessation of PFA feeding at both doses by 19.7% ($P<0.002$) and 6.4%, respectively. Increasing the dose of PFA did not increase the daily gain of calves, but an increase in the level of SBA was recorded. However, the obtained data indicated that the use of a double dose of probiotics contributed to a reduction in the incidence of calves with symptoms of diarrhoea by 50% and 33%, respectively, in the I and II experimental groups. At the same time, among the control calves, the incidence of animals with gastrointestinal symptoms treated with antibiotics for 4–7 days was 60%. Increased body temperature to 39.5°C was

Table 2. Clinical indicators in calves fed PFA D1. Data are presented as mean±SD

Group (n=10)	D1 dose	Days of application	LWG, kg	Morbidity, %	SBA, %	
					Before use	After use
Newborn calves; stage I						
Experimental 1	5 g	10	0.738±0.042**	30	24.03±3.05	38.09±0.24**
Experimental 2	10 g	10	0.735±0.024**	20	19.80±0.71	33.85±3.26***
Control	–	–	0.642±0.019	60	22.08±3.52	31.82±4.46
One-month-old calves; stage III						
Experimental 1	5 g	1–20	0.716±0.07	30	33.21±1.93	44.97±2.94
Control 1	–	–	0.680±0.05	90	32.44±2.01	34.12±1.23
Experimental 2	10 g	21–40	0.882±0.065**	10	44.97±2.94	43.22±2.94**
Control 2	–	–	0.762±0.054	40	32.44±2.01	39.84±7.76

LWG= average daily live weight gain; SBA=serum bactericidal activity; *P<0.033; **P<0.002; ***P<0.001.

Table 3. Blood serum biochemical indicators in newborn calves fed PFA D1 (Experimental 1; Experimental 2; Control) and calves fed PFA D1 for 60 days (Experimental 3 and Control 3). Data are presented as mean±SD (n=10)

Indicator	Groups of animals				
	Experimental 1	Experimental 2	Control	Experimental 3	Control 3
Glucose, mmol/L	289±0.09*	3.18±0.13**	2.64±0.17	2.67±0.29	2.88±0.17
Total protein, g/L	64.72±1.33**	65.59±0.97**	58.06±2.58	68.75±1.32	67.06±2.58
Albumin, g/L	34.11±0.85	36.64±2.16	33.74±1.39	37.07±0.83*	33.74±1.39
Urea, mmol/L	3.75±0.19	3.77±0.12	3.68±0.31	3.05±0.39	2.98±0.31
AST, U/L	61.43±4.79	61.15±2.31	60.82±3.15	54.43±4.79*	60.82±3.15
ALT, U/L	20.13±1.04	19.21±1.07	20.08±0.89	12.38±1.04*	14.18±0.89
GGT, U/L	4.27±0.28	4.30±0.11	4.26±0.15	2.24±0.78	2.26±0.71
Calcium, mmol/L	2.53±0.05*	2.85±0.16*	2.24±0.09	2.57±0.05	2.54±0.09
Phosphorus, mmol/L	2.89±0.12*	2.74±0.17*	2.42±0.08	2.89±0.12*	2.62±0.08

*P<0.002; **P<0.001.

Table 4. Clinical indicators in 14-day-old calves fed PFA L1 and L2. Data are presented as mean±SD

Group (n=10)	PFA	LWG, kg	Morbidity, %	SBA, %	
				Before use	After use
Stage II					
Experimental 1	L1	0.53±0.047**	30	25.04±2.75	33.75±0.23**
Experimental 2	L2	0.67±0.056**	30	24.80±0.55	34.16±3.26**
Control	–	0.46±0.054	60	21.12±2.12	29.76±3.14

LWG= average daily live weight gain; SBA=serum bactericidal activity; **P<0.002.

registered in calves with diarrhoea, treated with antibiotics. The calves recovered on the 5th day of antibiotic therapy. In the cases when during the disease body temperature exceeded 39.6 °C, the antibiotic therapy was extended to 7–10 days. Thus, the use of PFA D1 in newborn calves prevented the use of at least 35 daily courses of antibiotic therapy in the neonatal period. After the cessation of PFA feeding, a tendency toward increase of blood serum glucose total protein; total calcium and inorganic phosphorus was recorded in calves from the 1 and 2 experimental groups relative to the control values (Table 3).

Stage II – 14 days old calves (n=30)

PFA L1 and L2 were given at a dose of 10 mL per animal/day with milk. After 12-day feeding period, the average daily gain was determined and the diseases of calves

with symptoms of damage to the gastrointestinal tract were recorded.

In the calves of the first experimental group, the mean LWG was higher than the control by 70 g, whereas in the second experimental group by 210 g (by 15.2% and 45.6% higher, respectively). Therefore, the use of PFA containing yeasts, contributed to a more intensive increase in the live weight of calves. In the animals of the experimental groups, the SBA indicator increased by 34.7% and 37.7% in the first and second experimental groups ($P<0.002$) in comparison with the control group (Table 4). It was established that PFA had a pronounced prophylactic effect on diarrhoea in calves. Thus, during the observation period, digestive disorders were registered in 6 calves in the control group, and three animals fell ill in each of the experimental groups, which recovered after 3–5 days of application of the traditional treatment scheme for the farm. Thus, the use of PFA L1 and L2 in 14-day-old calves prevented the use of at least 30 daily courses of antibiotic therapy in calves in the neonatal period.

Stage III – one-month-old calves (n=20)

The results indicated that the use of PFA D1 for 20 days did not significantly increase LWG in calves compared to the control group. At the same time, the level of SBA in calves of the experimental group was higher ($P<0.001$) in comparison to its control values (Table 2).

Later, after another 40 days of PFA application at a dose of 10 g per animal/day, a tendency to decrease in SBA was recorded along with an increase in the LWG ($P<0.002$) compared to the control group (Table 2). Thus, the daily application of PFA D1 for 2 months did not provide a sufficiently stable level of SBA, against the background of a steady increase in the LWG in calves relative to the control. The results showed that digestive system disorders and signs of rhinitis were registered among the experimental groups during the first observation period. The incidence rate of calves in the control group was 90%, and in the experimental group: 30%. Later, during the next 40-day observation period, the calves showed upper respiratory tract infections, sometimes with bronchopneumonia (10% and 40% in the experimental and control groups, respectively). Empiric use of antibiotics for 4–7 days was applied in these calves. Thus, the use of PFA D1 prevented the use of at least 45 daily courses of antibiotic therapy in calves of experimental groups. An increase in serum albumin and inorganic phosphorus ($P<0.002$) was recorded in calves from experimental group 3 (Table 3). The increase in the albumin content was recorded against the background of the absence of changes in the amount of total protein, indicating a decrease in total globulins content along with a decrease ($P<0.002$) in the activity of AST and ALT (Table 3). The determined physiological values of the activity of hepatospecific enzyme GGT and the content of urea illustrated the absence of toxic and/or pathophysiological effects of PFA on this age group as well.

Stage IV – calves aged 2 and 3 months (n=40)

In the calves from the 1st ($P<0.002$) and 2nd ($P<0.001$) experimental groups, the LWG exceeded the control level (Table 5). The SBA index in experimental calves after 21-day PFA application changed in a multidirectional manner. In 2-month-old calves (experimental group 1), the indicator level was lower than the control, and in 3-month-old calves (experimental group 2), it was increased ($P<0.002$).

Table 5. Clinical indicators of 2-month-old (Experimental 1) and 3-month-old (Experimental 2) calves fed PFA L2. Data are presented as mean±SD

Group (n=10)	L2 dose	Days of application	LWG, kg	Morbidity, %	SBA, %	
					Before use	After use
Stage IV						
Experimental 1	10 mL	21	0.777±0.11*	50	33.23±1.91	38.84±2.50
Control 1	–	–	0.666±0.07	40	32.40±2.01	45.21±9.1
Experimental 2	10 mL	21	0.840±0.04**	30	44.90±2.94	50.14±1.71**
Control 2	–	–	0.691±0.04	40	36.02±1.88	46.11±2.23

LWG= average daily live weight gain; SBA=serum bactericidal activity; *P<0.002; **P<0.001.

Table 6. Blood serum biochemical parameters in 2-month-old (Experimental 1; Control 1) and 3-month-old (Experimental 2; Control 2) calves fed PFA L2 for 21 days and of 4-month-old calves fed PFA D1 (Experimental 3; Control) and PFA D2 (Experimental 4; Control) for 14 days. Data are presented as mean±SD (n=10)

	Experimental 1	Control 1	Experimental 2	Control 2
Glucose, mmol/L	2.92±0.29*	2.60±0.13	2.98±0.53*	2.64±0.17
Total protein, g/L	68.77±1.35*	59.83±1.74	69.13±1.17**	61.06±2.58
Albumin, g/L	37.11±0.81*	32.86±0.95	38.45±0.59**	33.74±1.39
Urea, mmol/L	3.25±0.19	3.38±0.11	3.31±0.17	3.68±0.31
AST, U/L	61.43±4.79	59.48±2.03	62.43±1.21	60.82±3.15
ALT, U/L	15.30±1.04	14.27±0.61	16.22±0.74	14.18±0.89
GGT, U/L	2.71±0.18	2.58±0.13	2.75±0.12	2.56±0.71
Calcium, mmol/L	2.56±0.05	2.51±0.07	2.55±0.08	2.54±0.09
Phosphorus, mmol/L	2.81±0.12*	2.49±0.06	2.83±0.12**	2.52±0.08

	Experimental 3	Experimental 4	Control
Glucose, mmol/L	2.50±0.14**	3.13±0.15**	1.57±0.16
Total protein, g/L	80.25±2.03	82.76±1.48*	72.09±1.64
Albumin, g/L	33.82±2.51	35.61±0.79	32.61±1.91
Urea, mmol/L	5.28±0.11*	4.57±0.26**	6.53±0.73
AST, U/L	66.55±1.27**	64.58±2.12**	82.55±6.59
ALT, U/L	21.56±2.21	22.78±1.64	19.58±2.08
GGT, U/L	1.84±0.21	1.87±0.51	2.02±0.19
Calcium, mmol/L	2.74±0.16	2.89±0.11**	2.62±0.07
Phosphorus, mmol/L	1.79±0.07*	1.99±0.12**	1.54±0.11

*P<0.002; **P<0.001.

The results from observations of the clinical condition of the animals of the experimental and control groups indicated that the disease level of calves with symptoms of respiratory tract damage was similar and ranged between 40–50% in 2-month-old calves and 30-40% in 3-month-old calves. That is, in this study, the use of PFA L2 contributed to the reduction of morbidity only among 3-month-old calves and prevented the use of a 5-day course of antibiotic therapy. The performed blood serum biochemical tests (Table 6) indicated that 21-day administration of PFA to 2-month-old (Experimental group 1) and 3-month-old (Experimental group 2) calves led to significant increase in glucose, total protein along with their albumin fraction and inorganic phosphorus, relative to the values in control groups 1 and 2.

Stage V – calves aged 4 months (n=30)

The calculation of LWG showed a higher daily gain in animals of experimental group 4, which received the drug D2 compared to the control (P<0.002, Table 7).

The SBA indicator was higher in animals of the second experimental group compared to the indicator in the control group. Clinical observations showed that the calves with respiratory signs in the first group were 40%, in the second group: 30% whereas in the control group, the disease level was 50%. Thus, the application of PFA D1 to 4-month-old calves prevented the use of at least 5 daily

Table 7. Clinical indicators of 4-month-old calves fed PFA D1 (Experimental 3) and D2 (Experimental 4). Data are presented as mean±SD

Group (n=10)	PFA, dose	Days of application	LWG, kg	Morbidity, %	SBA, %	
					Before use	After use
Stage V						
Experimental 3	D1, 10 g	14	0.525±0.041	40	36.02±1.73	42.14±2.34
Experimental 4	D2, 10 g	14	0.537±0.008*	30	35.52±1.41	44.24±1.34
Control	_	–	0.480±0.045	50	36.11±1.32	40.14±2.34

LWG= average daily live weight gain; SBA=serum bactericidal activity; *P<0.002.

Table 8. Average daily increase in live weight of heifers fed D2 for 21 days. Data are presented as mean±SD

Group (n=10)	PFA, dose	LWG, kg	
		Before use	After use
Stage VI			
Experimental 1	D2, 40 g	0.360 ± 0.010	0.516 ± 0.010**
Experimental 2	D2, 20 g	0.351 ± 0.011	0.401 ± 0.012*
Control	–	0.344 ± 0.010	0.346 ± 0.011

LWG= average daily live weight gain; *P<0.002; **P<0.001.

courses of antibiotic therapy, and the use of PFA D2 – 10 daily courses of antibiotic therapy, respectively.

Table 6 shows the results from assays of the main blood serum biochemical indicators in calves before (control) and after feeding PFA of different variants (Experimental group 3; Experimental group 4). It was established that after feeding PFA D1 (Experimental 3) the content of glucose and phosphorus in the blood serum of calves increased along with a decrease in the level of urea and AST activity (P<0.001; P<0.002), compared to the control values. At the same time, in the calves of the second experimental group, the use of PFA D2 (Experimental 4) resulted in increased total proteins and calcium (P<0.001; P<0.002), along with an increase in the level of glucose and phosphorus (P<0.001; P<0.002), when compared to the respective control values. At the same time, the values of urea and AST activity also decreased (P<0.001).

Both variants of PFA contributed to the restoration of metabolic processes and reduction of the toxic load in young cattle relative to control animals and corresponded to the state of clinical indicators. However, in animals of the control group, a decrease in the level of glucose and phosphorus on average by 47.7% and by 13.5% (P<0.001) was recorded as well as an increased activity of AST and ALT – on average by 175.2% and 97.9% (P<0.001) respectively, relative to the reference values of indicators (glucose 2.5–3.5 mmol/L, phosphorus 1.80–2.50 mmol/L, AST 10–50 U/L, ALT 10–30 U/L) (Table 6). Therefore, the use of probiotics in the experimental calf rearing system contributed to the recovery of the indicated indicators, namely: the content of glucose and phosphorus in the blood serum of animals treated with PFA D1 and PFA D2 increased relative to that in the controls by an average of 59.2 and 99.4% and by 16.2 and 29.2% (P<0.001); while the activity of AST and ALT decreased by 19.4 and 21.8% and by 45.5 and 42.4% (P<0.001), respectively, indicating slowing down of peramination processes.

The obtained results indicated a positive effect of the proposed treatment on the restoration of metabolic processes along with the activity of the endogenous detoxification function of the liver of experimental cows obviously due to better assimilation of nutrients from feed by animals.

Stage VI – heifers aged 12 months (n=30)

The results from Table 8 showed that during this period the LWG in the 1st experimental group was by 43.3% (P<0.001) higher than in the control group, and in the 2nd experimental group – higher by only 14.2% (P<0.002). The obtained results indicated that in this age group, a dose of 40 g per animal/day provided a higher level of increase in live weight of heifers. During the observation period, diseases among the animals involved in the experiment were not registered.

DISCUSSION

The results allowed evaluating the influence of PFA on the clinical condition and live weight gain of calves. The use of probiotics in livestock feed is justified due to the ban on the use of antibiotics in feed by the European Union (Ogbuewu *et al.,* 2018).

The results of our research on the use of a feed additive containing live probiotic cultures of *Bacillus subtilis* and *Bacillus*

licheniformis for calves are in line with the published literature data. According to some authors (Lytvyn & Polishchuk, 1990; 2021), feed additives containing the mentioned microorganisms showed good effect on the growth and development of calves and can be used in a double dose as therapeutic and preventive agents. The effect of probiotics on calves during the neonatal period was noticeable even when cows were fed PFA before calving (Kritas *et al.*, 2006). Feeding probiotic cultures of *Bacillus subtilis* and *Bacillus licheniformis* directly to newborn calves improved twice the growth performance (Koike *et al.*, 2021; Lucey *et al.*, 2021). It is acknowledged that the symbiosis of these microorganisms has a therapeutic and preventive effect on indigestion in calves and helps stopping the symptoms of diarrhoea in the neonatal period. Our results also indicate that the use of PFA, containing *Bacillus subtilis* and *Bacillus licheniformis* to newborn calves at a dose of 10 g per animal/day provided a pronounced preventive and therapeutic effect for digestive disorders in calves; and the use of the drug at a dose of 5 g per animal/day helped improving the growth and physiological development of animals. There was also a tendency towards increase in albumin and urea content in the calves of the experimental groups, which, together with the above-mentioned changes in the indicators of protein, carbohydrate and mineral metabolism in the body of calves, indicated an enhanced activity of metabolic reactions due to the use of both doses of probiotic supplements. Physiological values of the activity of hepatospecific enzymes – ALT, AST and GGT, were determined to illustrate the absence of toxic and/or pathophysiological effects of PFA on the body of newborn animals. Data (Wang *et al.*, 2016) show that the use of *Bacillus licheniformis* promotes the fermentation of roughage. The results obtained by us in experiments with older calves also indirectly confirmed the data of these authors.

It has been proven (Villot *et al.*, 2019) that the use of live yeast supplements modulates the microbial composition of the intestinal contents and increases the level of resistance, prevents diarrhoea in dairy calves. Calves that received the supplement were less sick, had a lower percentage of severe diarrhoea (9.5 vs. 28.6%) and recovered faster when forced to use antibiotics. According to Cangiano *et al.* (2020), the positive effect of using probiotics in calves from birth to one week of age is associated with an increase in the synthesis of secretory IgA in the small and large intestines. However, as evidenced by Pisoni & Relling (2020), the use of yeast supplements did not have a therapeutic effect during a severe course of diarrhoea. In older calves, the use of yeasts helped to reduce the level of respiratory diseases (Klopp *et al.*, 2022). Some authors (Jensen *et al.*, 2008; Brewera *et al.*, 2014) indicated that the addition of *Sacharomyces cerevisiae* to the feed for older dairy calves had an anti-inflammatory and immunostimulating effect, in particular due to the prevention of intestinal colonisation by suppressing growth of *E. coli*. The results of our research showed that the use of yeast in the PFA composition contributed to an increase in the live weight of calves in all age groups, confirming data of Takemura *et al.* (2020) that feeding *S. serevisiae* to calves contributed to a more intensive increase in live weight in calves older than 3 weeks of age. In literary sources, the debate regarding the influence of *S. serevisiae* on the development of rumen in ruminants still continues. There is evidence (Richard *et*

al., 1990) that the accumulation of end products of fermentation inhibits the level of feed intake. Other authors (Jennifer & Laborde, 2008) claim that the addition of *S. cerevisiae*, *B. subtilis* and *B. licheniformis* to grain diets did not affect the development of the rumen. At the same time, Graham & Simmons (2005) and Xiao *et al.* (2016) indicated that fermentation products of *S. serevisiae*, fed to calves before weaning with milk, improved the morphology of the rumen. Batista *et al.* (2022) showed that the use of yeast products contributed to increasing the productivity of ruminants and improving blood parameters.

Our studies indirectly indicated that PFA containing *Sacharomyces cerevisiae* AF 338 contributed to the increase in the live weight gain in calves and the level of natural resistance. The effect of PFA from *Sacharomyces cerevisiae* on the dynamics of the SBA was however different: in 2-month-old calves, it decreased by 14.1% in relation to the control group, so the influence of yeasts on the natural resistance of calves of different sex and age groups needs additional study. The administration of PFA D2 to calves at the age of 3 months did not affect negatively the growth and development of the animals. The obtained data indirectly confirm the results of another study (Opsi *et al.*, 2011) regarding the pronounced positive effect of live yeast on fermentation and fermentative processes in the rumen (Gibson *et al.*, 2017), which in turn is reflected in live weight gain.

Application of PFA D2 to 12-month-old heifers did not have a pronounced stimulating effect on live weight gain, but no negative effect was either registered. We suggest further studies on effective ways of applying PFA to heifers of premating age in order to determine the impact on the level of fertilisation and homeostasis of offspring. In addition, one of the directions of further research may be the study on the effectiveness of the use of probiotics for prevention of metabolic diseases in high-yielding cows.

According to Liu *et al.* (2018), the effectiveness of feed additives containing yeasts explains their high antioxidant activity. It is necessary to take into account provided evidence (Ogbuewu *et al.*, 2018; Lytvynenko & Yukhymchuk, 2021) that the efficiency of this effect on the scar microbiome cannot be constant in the long term.

It should be noted that the use of PFA contributed to a decrease in the level of morbidity in calves, which in turn contributed to a decrease in the level of use of antibiotics, which were part of the therapy schemes in this farm. It can be therefore stated that the use of probiotics (in our case – in the form of a feed additive containing strains of *Bacillus subtilis* 12P-130, *Bacillus licheniformis* 12P-896 and *Sacharomyces serevisiae* AF 338) is possible for prevention of the occurrence of diseases in calves, as an available alternative to the use of antibiotics for the treatment of diseases of young animals in the complex therapy schemes. According to our observations, the treatment of one calf lasts 5 days (that is, 5 days of antibiotic therapy). Prevention of disease of one calf thus means prevention of 5 days of antibiotic therapy. In other words, the use of probiotics is an experimentally justified measure within the framework of the introduction of a systemic fight against the spread of antibiotic resistance in pathogenic microorganisms.

In general, the results of blood biochemical studies of the animals that received probiotic feed additives, indicated an increase in metabolic reactions in the

body of young cattle, which is an illustration of the predominance of anabolic over catabolic processes, and can provide a general strengthening of their health. The use of PFA contributes to the stabilisation of metabolic, energy and processes of excretion of protein residues from the body and antitoxicant activity of the liver in experimental calves, mitigation of feed stress and increase in the level of resistance.

CONCLUSION

The use of the tested probiotic feed additive (*Bacillus subtilis* 12P-130, *Bacillus licheniformis* 12P-896 and *Sacharomyces cerevisiae* AF 338) at all stages of raising calves contributed to increasing the economic efficiency of the livestock industry, reducing the level of use of antibacterial agents and obtaining ecological products; confirming the possibility of using probiotics as an alternative to antibiotics.

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