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EFFECT OF MANNANOLIGOSACCHARIDES, INULIN AND YEAST NUCLEOTIDES ADDED TO CALF MILKREPLACERS ON RUMEN MICROFLORA, LEVEL OF SERUM IMMUNOGLOBULIN AND HEALTH CONDITION OF CALVES

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ABSTRACT

The aim of study was to determine effect of applied in calf milk replacer feed additives – mannanooligosaccharides, inulin and yeast nucleotides on rumen microflora, level of serum immunoglobulin and calf health condition. The results of research related to effect of mannanooligosaccharides, inulin and yeast nucleotides added to calf milk replacers on calf rearing and health condition point to higher final body weight as well as higher daily body weight gains, better concentrate intake and feed conversion ratio all rearing long. Feed additives, especially yeast nucleotides had beneficial influence on faeces scores – better consistency (less watery and well formed). It can testify to better calf health condition. Additives applied in calf milk replacers did not clearly affect on morphological and biochemical blood and serum parameters. Merely blood glucose increased while levels of cholesterol and plasma urea N were reduced. The higher level gamma-globulin as well as better passive immunity transfer were stated in calves receiving mannanooligosaccharides and yeast nucleotides in amount 4 g/day/head in milk replacer. Applied in calf milk repalcers mannanooligosaccharides, inulin and yeast nucleotides, especially inulin in amount 6 g/day/head increased calf rumen pH and decreased level of rumen ammonium nitrogen. Concentration of total volatile fatty acids in rumen, especially acetate and propionate were higher in calves receiving in milk replacer prebiotic feed additives, especially mannanooligosaccharides and yeast nucleotides what was confirmed by higher body weight gains. Moreover the total bacteria count increased while the concentration of protozoa in the rumen fluid decreased, particularly in calves receiving inulin.

Key words: mannanoligosaccharides, inulin, yeast nucleotides, calves, rumen microflora, blood, immunoglobulin, growth performance, health condition

INTRODUCTION

High mortality of calves, caused by their poor immunity as well as bacterial infections in digestive and respiratory tracts, made using of feed antibiotics in calf milk replacer happened. Currently, in order to reinforce of calves' resistance to pathological agents scientists are still researching alternative compounds and substances – feed immunomodulators that are more and more using in feed industry. Prebiotics are ranked among those compounds. They are described as non-digestible food ingredients that stimulate the growth and/or activity of microorganisms in the dige-

stive system [17]. Among prebiotics mainly non-digestible oligosaccharides are ranked: fructooligosaccharides (FOS), mannanooligosaccharides (MOS), and galactooligosaccharides [19,21]. Beneficial bacteria (lactic acid fermentation) have enzymatic complex essential to utilization of those oligosaccharides. By virtue of above beneficial changes in microbial homeostasis of intestine are observed [4,33,35]. Oligosaccharides lengthen intestinal villuses and increase production of short-chain fatty acids. Oligosaccharides not only prevent from adhesion of pathogens to intestinal mucosal. They also stimulate growth and activity of beneficial bacteria and depress proliferation of pathogens in digestive tract and prevent from harmful bacteria adhesion to intestinal mucosal by agglutination of gram negative bacteria. In own researches on calves the research subjects were three prebiotics: mannanooligosaccharides, inulin and yeast nucleotides.

Mannaooligosaccharides (MOS) – complex carbohydrates containing cell walls of *Saccharomyces cerevisiae* yeast. Proposed way of action is based on mannanooligosaccharides ability to agglutination of specific gram negative bacteria by interaction with mannose-sensitive lectins located to these bacteria surface. They provide competitive bounding places for intestinal pathogens. Most of gram negative bacteria attach to intestinal epithelium using specific mannose-fimbriae [35,39]. It makes that mannans on bacteria surface are important antigen yeast cells [3]. Numerous strains of *Escherichia coli* and *Salmonella* are attached to MOS *in vitro* [39]. Mannanooligosaccharides support pathogens recognition by cells of immune system in intestine what improve local immunity.

Inulin is a polysaccharide consists fructose joined by a beta 2,1 glycosidic bond containig small amounts of glucose (one unit of glucose and ≤ 60 fructose units). Inulin is neither digested nor absorbed in small intestine but it is selectively and quickly fermented by bacteria in further parts of alimentary tract stimulating proliferation of lactobacillus, mainly *Bifidobacterium*. Bifidogenic action mechanism is based on selective fermentation of fructans by bifidobacteria synthesing beta-fructosidasis, enzyme decomposing beta 2,1 glycosidic bonds in inulin and oligofructosis [17]. Change of bacterial microflora in intestine, consisted in depressing number of harmful bacteria is observed as a result of bifidogenic effect. Their proliferation is inhibited by bifidobacteria that produce short-chain fatty acids (SCFA) and lower pH of intestine chyme the same bring about adverse conditions for pathogens. Moreover bifidobacteria compete with pathogens for adhesion's place in intestinal epithelium, for nutrients and produce antibiotic substances, so called bacteriocins and hydrogen peroxide Among species that proliferation is depressed by various strains of bifidobacteria are belonged to among others *E. coli, Salmonella, Shigella, Campylobacter jejuni* and *Clostridium perfirgens*. During bacterial fermentation of fructans short-chain fatty acids are produced, especially acetic, propionic, lactic and butyric acid [17]. These acids show beneficial effect on metabolism, nourish intestinal cells, lower pH of intestinal chyme and lengthen intestinal villuses as well as increase number of epithelial cells in particular villus.

Nucleotides play important role in metabolism and energy conversion in animal. They stabilize beneficial microflora of intestine and favourably effect on intestine tissue structure, improve nutrients absorption efficacy, prevent from diarrhea and in result affect better feed conversion and higher body weight gains [46]. Most of using in animal nutrition feeds contain insufficient amount of these compounds. Nucleotides in feeds show immunostimulative properties related to both cellular and humoral immunity. Such an action mechanism has not been interchangeably clarified. It seems that it could be connected with high requirement of intensively proliferated immunological cells for nucleotides in this connection endogenous synthesis in some cases is not sufficient [18]. Applied in animal nutrition nucleotides are *Saccharomyces cerevisiae* yeast origin. They are composed of yeast extracted natural RNA, nucleotides, nucleosides (nucleotides' precursor), organic acids, vitamins and termolised yeast as a carrier. With regard to immunostimulative properties nucleotides are particularly important in newborn and growing animal which immunity system is not fully mature [9]. Requirement for nucleotides increase during stress, intensified growth and in after weaning period. Intestine is an organ with big immunological capability and providing nucleotides affect decreases number of alimentary tact diseases [24].

Prebiotics cause potential possibilities of their practical using in non-specific immunoprophylaxis lots of animal diseases. The ban placed on using antibiotic growth promoters causes prebiotics could be alternative solution in cattle, swine and poultry farms. They also could be use in various infection prophylaxis and treatment by virtue of their efficacious mobilization of animal immune system and in consequence protect them from being afflicted with various contagions. Additives containing prebiotics could be used to correct often stated in animals defective immunity and immunological deficiency.

The aim of study was to determine effect of applied in calf milkreplacer feed additives – mannanooligosaccharides, inulin and yeast nucleotides on rumen microflora, level of serum immunoglobulin and calf health condition.

MATERIAL AND METHODS

The experiments were carried out in autumn-winter season at the turn of the 2005/2006, 2006/2007, 2007/2008 in dairy cow Farm Mlekoland in Przecza. Research material was consisted of 108 cow calves Holstein-Friesian breed. Animals were kept in individual boxes on deep straw and permanent access to water. Three experiment on calves were carried out. In each experiment animals were randomly assigned to 3 feeding groups, 12 calves each. All animals after lasting three days colostrum feeding period until 14 day if life were fed whole milk.

After lasting three days transitional period when calves received whole milk and milk replacer in the ratio of 1 to 1, milk replacers were given to animals in amount 10L/day/head, 5L each feeding (at 07:00 AM and 03.00 PM). From third day of life calves received concentrate mixture and water *ad libitum*. Milk replacer and concentrate mixture components were chemically analyzed and basic nutrients were determined – dry matter, crude ash, crude protein, ether extract as well as crude fiber – according to valid methods [1] (Table 1).

Table 1. Nutrients content in 1kg DM feeds used during calf rearing

Item	Unit	Milk replacer	Concentrate mixture
Gross energy	MJ/kg	16.8	6.75
	kcal/kg	4 015	1 613
Crude protein	%	21.0	24.0
Ether extract	%	16.0	3.4.
Crude fiber	%	0.05	5.5
Crude ash	%	7.0	7.3.
Nitragen free extractives	%	46.0	8.0
Calcium	g/kg	11.5	8.5
Phosphorus	g/kg	10.0	5.5
Sodium	g/kg	6.0	3.5

Feeds were given individually to calves and their intake were controlled daily. Agents differentiating feeding groups were added to milk replacer mannanooligosaccharides (experiment I), inulin (experiment II) and yeast nucleotides (experiment III). In each of experiments calves in control groups received milk replacer use in livestock farm containing dried eggs and probiotic – *Enterococcus faecium* M74. In experimental groups mannanooligosaccharides in amount of 2 and 4 g/day/head (experiment I), inulin in amount 3 and 6 g/day/head (experiment II) and yeast nucleotides in amount 2 and 4 g/day/head (experiment III) were added to milk replacer. During experiment lasting daily feed intake and weekly individual body weight gains were controlled. Moreover daily estimation of fecal score was carried on according to Larson's scale [29].

The blood samples of calves were taken from jugular vein on the 2nd, 23rd and 56th day of life before morning feeding. The level of glucose, total cholesterol, urea as well as blood morphology: red blood cells, white blood cells, platelets, haemoglobin and hematocrit were determined. The level of gamma globulin was also determined by paper electrophoresis method.

At the end of experiments three hours after morning feeding six calves were chosen from each group and rumen liquid were taken in amount 150–200 ml using esophagus probe. In taken rumen liquid samples pH using pH/ISE meter Orion 710A, ammonium nitrogen [11] and volatile fatty acids using gas chromatograph Hewlett Packard as well as protozoa and bacteria number according to Zawadzki's method [47].

All obtained data were statically analyzed with one factor variance analysis using Statistica 7.2 software and significance of differences was estimated by means of Duncan's multiple interval test.

RESULTS

Experiment I – mannan oligosaccharides

Calves receiving mannan oligosaccharides in amount 2 and 4 g/day/head showed clearly higher ($P \leq 0.01$) body weight in 56 day of life than calves from control group (Table 2). It was confirmed by higher daily weight gains of calves that milk replacers were supplemented by mannanooligosaccharides (Table 2). Higher daily weight gains were obtained in calves receiving 4g of mannanoolisaccharides in calf milk replacer daily (gr II) than in animals from others group but significance of differences were confirmed only in group I (Table 2). Better ($P \leq 0.05$) feed conversion per 1 kg of body weight gains was stated in calves that received mannan oligosaccharides in amount 4 g/day/head than in calves from others group.

In all groups fecal score in first period of calf rearing (0–28 day) was clearly worse than in second period (28–56 day). It could be a result of feeding change in 14 day of their live from whole milk on milk replacer as well as immunological deficiency caused by finishing passive immunity and insufficient calf active immunity and occurs in 3–4 week of life. Obtained data related to fecal score indicated that applied to calf milk replacers mannanooligosaccharides, especially in amount 4 g/day/head improved faeces consistency (Fig. 1).

Table 2. Calf growth performance (experiment I)

Items	Feeding groups		
	I – control	II – 2 g/day/head MOS	III – 4 g/day/head MOS
	SD	SD	SD
Body weight:			
– after birth	44.46 ±4.09	45.29 ±4.06	44.71 ±4.26
– 56 day	72.33^{A} ±2.24	80.46^{B} ±2.18	79.33^{B} ±2.24
Average daily body weight gains [g/day]:			
	498^{a} ±82	628^{b} ±74	618^{b} ±71
Concentrate mixtures intake [g DM/day]:			
	479^{a} ±23	534 ±21	579^{b} ±54
Feed conversion [kg DM./kg body weight gains]:			
	3.59^{a} ±0.58	2.92^{a} ±0.27	2.83^{b} ±0.33
Mortality[head]:	0	0	0

a, b – P ≤ 0.05 – significant differences, A, B – P≤ 0.01 – high significant differences

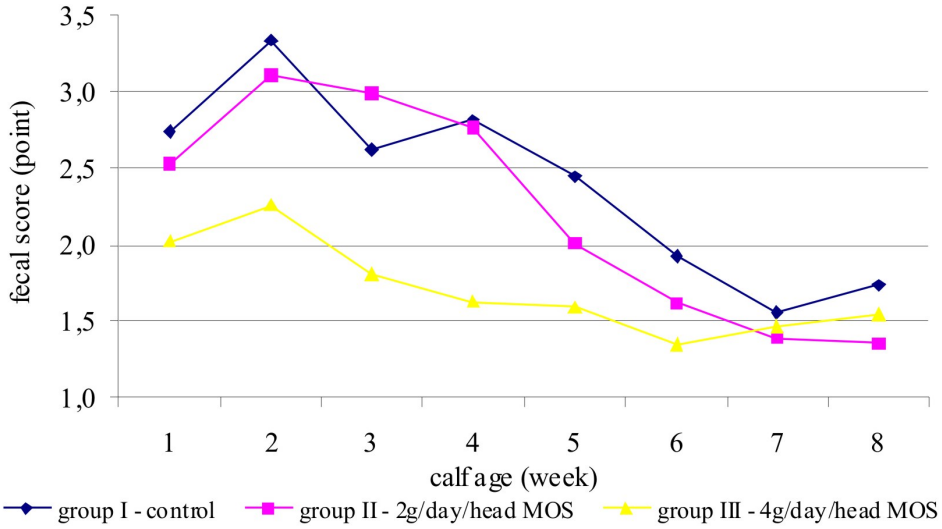

Fig. 1. Calf fecal score (experiment I)

The highest glucose content in blood in 2–4 day of calf life was shown in animals from control group while in 56 day of life higher (P≤0.05) glucose content was stated in calves from experimental groups (gr II and III).Urea level in serum all included in experiments calves was similar at the beginning of experiment though the highest urea level was stated in calf serum in group III In 56 day of life higher (P≤0.05) urea content in calf serum was noted in control group (gr I) than in calves from experimental groups (Table 3).

At the beginning of experiment (2–4 day) level of cholesterol and aspartate aminotransferase in calf serum in all groups was similar. In 56 day of life cholesterol level stated in calves that received in milk replacer mannanooligo-saccharides in amount 4 g/day/head (gr III) was lower (P≤0.05) than in calves from others group (Table 3).

White blood cell count in calves from all feeding groups in 2–4 day of their life was similar while in 56 day of life in calves that in calf milk repacer received mannanooligosaccharides in amount 2 g/day/head WBC count was higher (P≤0.05) than in others groups. In 56 day if calves life more than thrice higher platelets count was stated in animals that did not receive this prebiotic in milk replacer but by virtue of individual differences within experimental groups these differences were not statistically confirmed (Table 3). At the beginning (2–4 day) of experiment haemoglobin and hematocrit level in blood of calves from control group was higher (P≤0.05) than in animal from experimental groups (gr II and III). At 56 day of life haemoglobin and hematocrit level in blood of animals from control group (gr I) was significantly (P≤0.05) higher than in calves that milk replacer was supplemented by mannanooligosaccha-rides in amount 4 g/day/head (gr III).

Average gamma-globulin concentration amounted 11.59g×dm^{-3} in 2–4 day of life and 11.59g×dm^{-3} in 21–28 day. In 21–28 day gamma-globulin concentration in calf serum decreased and ranged from 6.57 to 7.18 g×dm^{-3} while in 56 day serum gamma-globulin content was higher (P≤0.05) in calves that received mannanooligosaccharies in milk replcer (Fig. 2). Higher rumen pH was stated in calves that manna oligosaccharides in amount 2 g/day/head (gr II) were given in milk replacer compared to animals that did not received this prebiotic (gr I). However significantly (P≤0.01) higher rumen pH value was noted in calves that this prebiotic was given in amount 4 g/day/head than in animals from control group.

Table 3. Biochemical parameters in serum and blood morphology (experiment I)

Items	Feeding groups		
	I – control	II – 2 g/day/head MOS	III – 4 g/day/head MOS
	$\bar{x}$ SD	$\bar{x}$ SD	$\bar{x}$ SD
Glucose [mg×dL^{-1}]:			
– 2–4 day	87.08±12.64	84.03±11.14	83.94±10.67
– 56 day	72.40^{a}±5.51	77.44^{b}±7.25	79.44^{b}±7.68
Urea [mmolxdm^{-3}]:			
– 2–4 day	3.71±1.92	3.24±0.89	4.13±1.05
– 56 day	4.26^{a}±0.38	3.48^{b}±0.47	2.79^{b}±0.29
Total cholesterol [mmol×dm^{-3}]:			
– 2–4 day	2.10±1.17	1.89±0.54	1.88±0.99
– 56 day	3.16^{a}±0.66	2.73^{b}±0.34	2.33±0.37
AST [Uxdm^{-3}]:			
– 2–4 day	72.25±31.43	56.67±21.06	66.50±24.41
– 56 day	56.17±29.97	66.50±13.45	57.75±21.15
White blood cells [10^{9}×dm^{-3}]:			
– 2–4 day	7.19±0.95	6.59±1.91	7.30±0.75
– 56 day	10.91^{a}±1.91	9.83±2.15	8.97^{b}±0.82
Red blood cells [10^{12}×dm^{-3}]:			
– 2–4 day	8.32±1.03	8.25±0.78	8.70±0.88
– 56 day	8.22±1.98	9.71±0.83	9.40±1.00
Platelets [10^{9}×dm^{-3}]:			
– 2–4 day	266±90	371±90	250±53.63
– 56 day	1097±409	340±179	303±176
Haemoglobin [g×dL^{-1}]:			
– 2–4 day	8.09^{a}±0.80	7.54^{b}±1.09	8.14±1.01
– 56 day	7.66^{a}±1.09	9.18±1.01	10.07^{b}±1.05
Hematocrit [l×dm^{-3}]:			
– 2–4 day	0.298^{a}±0.05	0.262^{b}±0.04	0.272±0.05
– 56 day	0.222^{a}±0.07	0.301^{b}±0.04	0.308^{b}±0.07

a, b – P ≤ 0,05 – significant differences, A, B – P≤ 0.01 – high significant differences

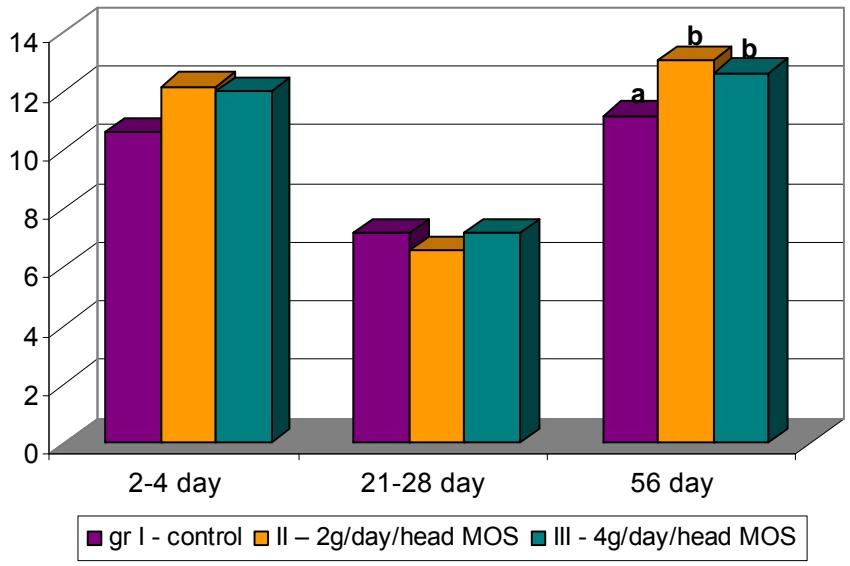

Fig. 2. Gamma-globulin concentration [g×dm^{-3}] in calf serum (experiment I)

a, b – P ≤ 0.05 – significant differences

The highest ammonium nitrogen level was stated in rumen fluid of calves from control group (without mannanooligosaccharides addition). This level was significantly (P≤0.05) higher than in rumen fluid of calves that received this prebiotic in amount 4 g/day/head (Fig. 3).

Feeding calves with milk replacer containing mannan oligosaccharides in amount 2 and 4 g/day/head (gr II and III) increased (P≤0.05) volatile fatty acids (VFA) concentration in rumen fluid. The highest VFA concentration was stated in rumen fluid of calves that received 2 g/day/head mannanooligosaccharides while the lowest concentration was noted in control group (without this prebiotic addition) (Fig. 4).

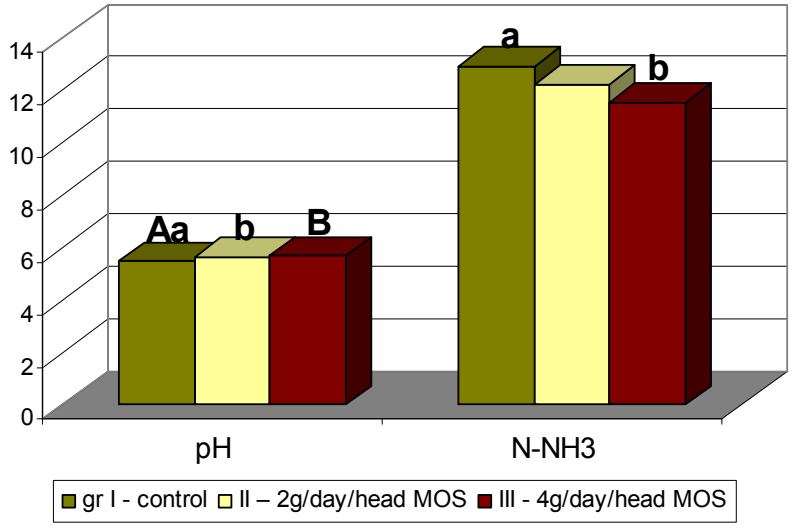

Fig. 3. Ammonium nitrogen [mg×dm^{-3}] and pH in calf rumen fluid (experiment I)

a, b – P ≤ 0.05 – significant differences, A, B – P≤ 0.01 – high significant differences

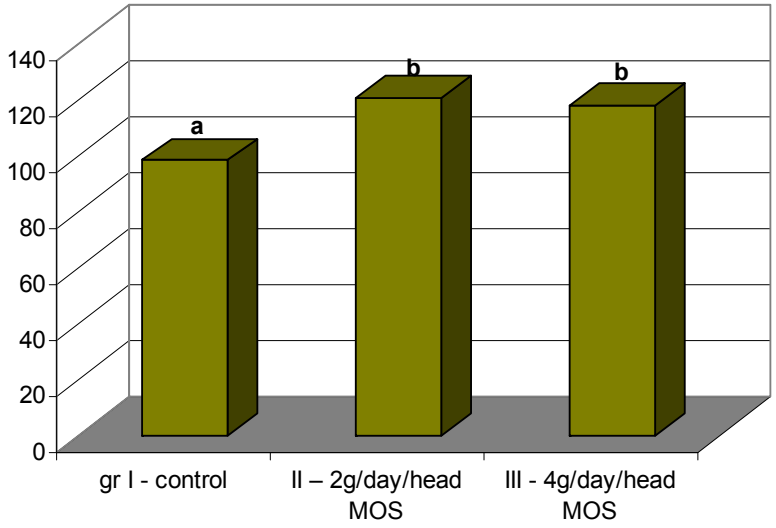

Fig. 4. Volatile fatty acids [mM×dm^{-3}] in calf rumen fluid (experiment I)

a, b – P ≤ 0.05 – significant differences

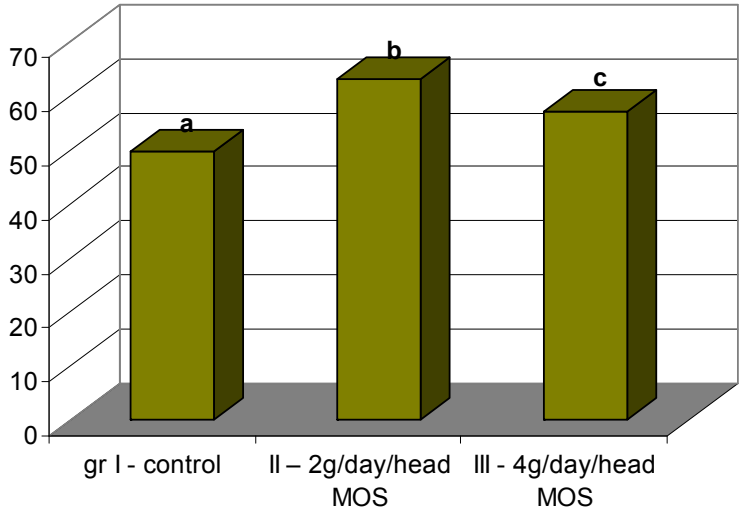

Fig. 5. Bacteria number [10^{9}] in calf rumen fluid (experiment I)

a, b, c – P ≤ 0.05 – significant differences

Mannanooligosaccharides applied in calf milk replacers indeed effected on bacteria number in rumen fluid (Fig. 5). The greatest bacteria number in 1ml of rumen fluid was stated in calves that received mannanooligosaccharides in amount 2 g/day/head and it was higher (P≤0.05) than bacteria number in rumen fluid of calves that did not receive mannan oligosaccharides (gr I) or received it in amount 4 g/day/head (gr III).

Applied in milk replacers mannanooligosaccharides significantly affected protozoa number in rumen fluid and their number was inversely proportional to number of bacteria in rumen fluid (Fig. 6). Number of protozoa in 1ml of rumen fluid of calves that did not receive mannanooligosaccharides was clearly (P≤0.01) higher than in calves that received this prebiotic in amount 2 and 4 g/day/head.

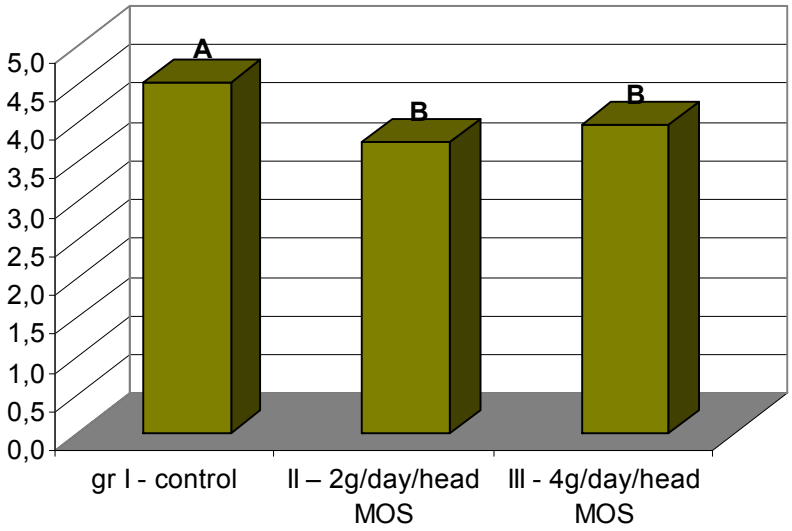

Fig. 6. Protozoa number [10^5] in calf rumen fluid (experiment I)

a, b – P ≤ 0.05 – significant differences

Experiment II – inulin

After birth body weight of calves was differential and significantly (P≤0.05) higher than in animal from group III. In 56 day calves that received inulin in amount 6 g/day/head (gr III) were shown clearly (P≤0.01; P≤0.05) higher final body weight than animals received this prebiotic in amount 3 g/day/head (gr II) and animals that did not receive inulin at all (gr I) It found affirmation in daily body weight gains and amounts of concentrate mixture intake. The highest values within these parameters were found in calves that inulin was administered in amount 6 g/day/head (gr III) but difference were not statistically confirmed. During calf rearing feed conversion [1 kg DM] per 1 kg body weight gains was better in calves from experimental group (gr II and III) that received inulin in amount 3 and 6 g/day/head (Table 4).

Table 4. Calf growth performance (experiment II)

Items	Feeding groups		
	I – control	II – 3g/day/head inulin	III – 6g/day/head inulin
	$\bar{x}$ SD	$\bar{x}$ SD	$\bar{x}$ SD
Body weight:			
– after birth	43.97^{a}±3.49	43.60^{a}±3.75	46.74^{b}±4.09
– 56 day	73.77^{a}±2.28.	70.69^{A}±1.91	78.72^{Bb}±2.27
Average daily body weight gains [g/day]:	552±78	479±71	572±73
Concentrate mixtures intake [g DM/day]:	490±24	444±18	500±46
Feed conversion [kg DM/kg body weight gains]:	3.40±0.55	3.27±0.51	3.00±0.41
Mortality[head]:	0	0	0

a, b – P ≤ 0.05 – significant differences, A, B – P≤0.01 – high significant differences

In first rearing period (0–28 day) calf fecal score in all groups was clearly worse than in second period of rearing (28–56 day). It could be an effect of feeding change in 14 day of calf life as well as immunological deficiency in 3–4 week of their life. Results of fecal score showed that inulin applied in calf milk replacers did not unambiguous effect on animal faeces. Consistency of faeces was slightly better in experimental group (gr II and III) (Fig. 7).

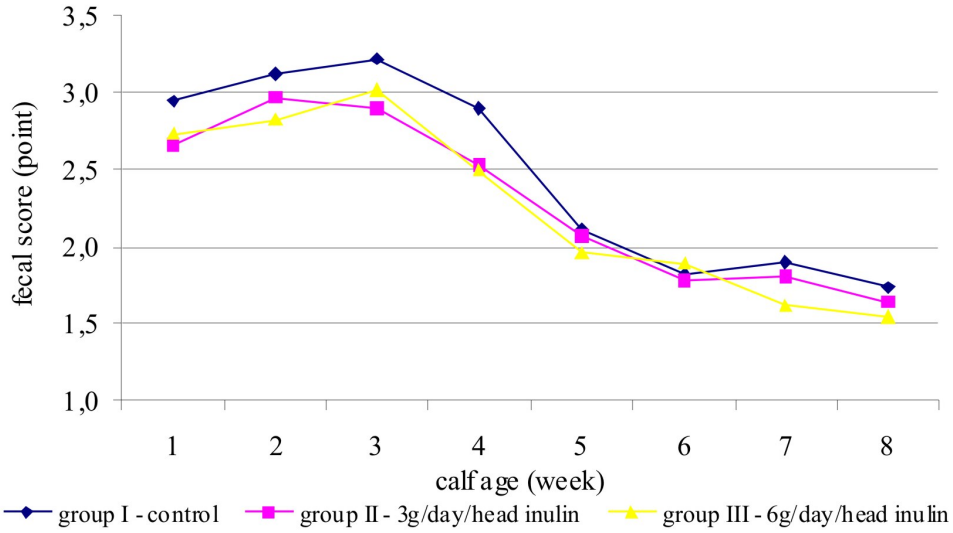

Fig. 7. Calf fecal score (experiment II)

Glucose content found in calf blood from control group in 2–4 day of life was higher (P≤0.05) than in experimental groups (gr II and III). In 56 day of calf live the highest glucose level in blood was noted in calves that milk replacers was supplemented by inulin in amount 3 g/day/head (Table 5). At the beginning of experiments serum urea content in all groups was similar while in 56 day of life urea level in calves from control group was clearly (P≤0.01) (P≤0.05) higher than in calves that received inulin in amount both 3 and 6 g/day/head. At the beginning of experiment level of cholesterol and aspartate aminotransferase (AST) in calf serum was similar in calves from all groups. Inulin added to calf that milk replacers affected lower cholesterol content in calf serum compared to control group (Table 5). Level of cholesterol in calves that received inulin in amount 6 g/day/head was lower (P≤0.05) than in calves that received this prebiotic in amount 3 g/day/head. It could be an evidence that amount of inulin was also of importance related to cholesterol level in serum (Table 5).

Table 5. Biochemical parameters in serum and blood morphology (experiment II)

Items	Feeding groups		
	I – control	II – 3g/day/head inulin	III – 6g/day/head inulin
	$\bar{x}$ SD	$\bar{x}$ SD	$\bar{x}$ SD
Glucose [mg×dL^{-1}]:			
– 2–4 day	80.44^{a}±6.13	78.65^{a}±7.92	74.93^{b}±17.31
– 56 day	67.80±9.35	73.59±7.35	69.62±10.60
Urea [mmol×dm^{-3}]:			
– 2–4 day	4.82±1.86	5.06±1.40	4.98±1.38
– 56 day	5.96^{Aa}±1.43	3.88^{B}±0.98	4.75^{b}±1.08
Total cholesterol [mmol×dm^{-3}]:			
– 2–4 day	2.04±0.54	1.70±0.40	1.97±0.68
– 56 day	4.19^{A}±0.54	2.98^{Ba}±0.37	2.41^{Bb}±0.66
AST [U×dm^{-3}]:			
– 2–4 day	83.59±36.36	63.65±23.66	73.20±29.30
– 56 day	63.64±33.96	78.90±15.95	79.23±29.02
White blood cells [10^{9}×dm^{-3}]:			
– 2–4 day	7.17±0.95	6.68±1.94	6.80±0.70
– 56. day	10.46^{a}±1.83	9.55^{a}±2.13	10.78^{b}±1.7
Red blood cells [10^{12}×dm^{-3}]:			
– 2–4 day	8.49^{a}±1.06	8.10±1.13	7.24^{b}±0.97
– 56 day	8.89±2.16	8.87±0.97	8.90±2.00
Platelets [10^{9}×dm^{-3}]:			
– 2–4 day	403±136	368±89	415±89
– 56 day	387±145	255±145	659±246
Haemoglobin [g×dL^{-1}]:			
– 2–4 day	8.87±1.01	9.24±1.01	8.72±1.27
– 56 day	10.00^{a}±1.16	11.45^{b}±1.64	9.40^{a}±0.89
Hematocrit [l×dm^{-3}]:			
– 2–4 day	0.240±0.04	0.298±0.05	0.255±0.07
– 56 day	0.291±0.06	0.301±0.10	0.315±0.04

a, b – P ≤ 0.05 – significant differences, A, B – P≤ 0.01 – high significant differences

White cells count in blood of calves from all experimental group was similar in 2–4 day of life. Higher white blood cells count was stated in animals that received milk replacer supplemented by inulin in amount 6 g/day/head than in calves from others groups. In 2–4 day of life red blood cells count in calf blood was diversified – the highest one in calves in control group (gr I) and it was significantly higher than in animals from group III. Red blood cells count in calves from all experimental groups was similar and did not depend on applied to milk replacer prebiotic (Table 5). The lowest platelets count in calf blood was noted both in 2–4 and 56 day of live in animal that received inulin in amount 3 g/day/head in milk replacer while the highest platelets count in blood of animals those milk replacer was supplemented by inulin in amount 6 g/day/head. However those differences were not statistically confirmed because of individual variability (Table 5). Haemoglobin level in blood of all calves was similar at the beginning of experiment whereas haemoglobin level in 56 day in calves that received inulin in amount 3 g/day/head was higher ($P \leq 0.05$) than in calves from others groups. Hematocrit level both at beginning and at the end of experiment in calves from all groups was similar (Table 5).

All analyzed blood and serum biochemical and morphological parameters ranged within limits of reference values according to Winnicka [43].

In 2–4 day of life average gamma-globulin concentration in calves from all experimental groups ranged from 8.52 to 12.48g×dm^{-3}. Calves could suffer from partial failure passive transfer (PFPT) and be more subject to diseases. Gamma-globulin concentration in calf serum decreased in 21–28 day of their life (Fig. 8).

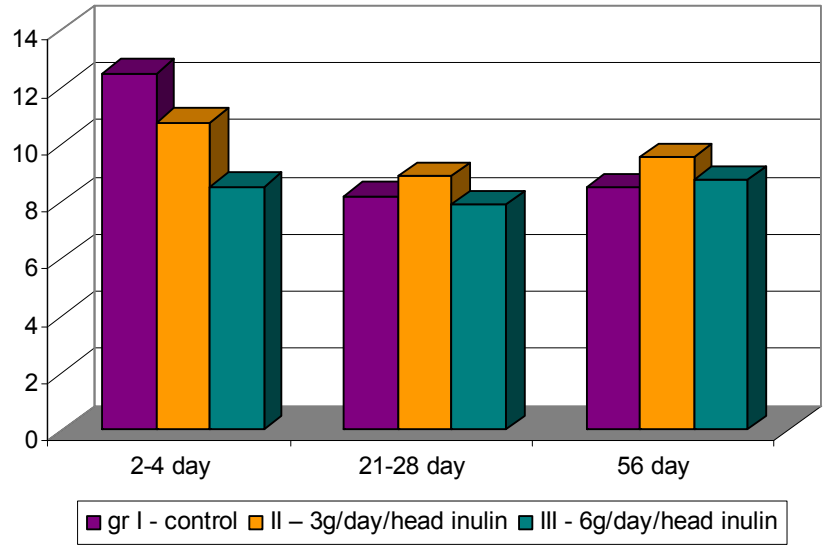

Fig. 8. Gamma-globulin concentration [g×dm^{-3}] in calf serum (experiment II)

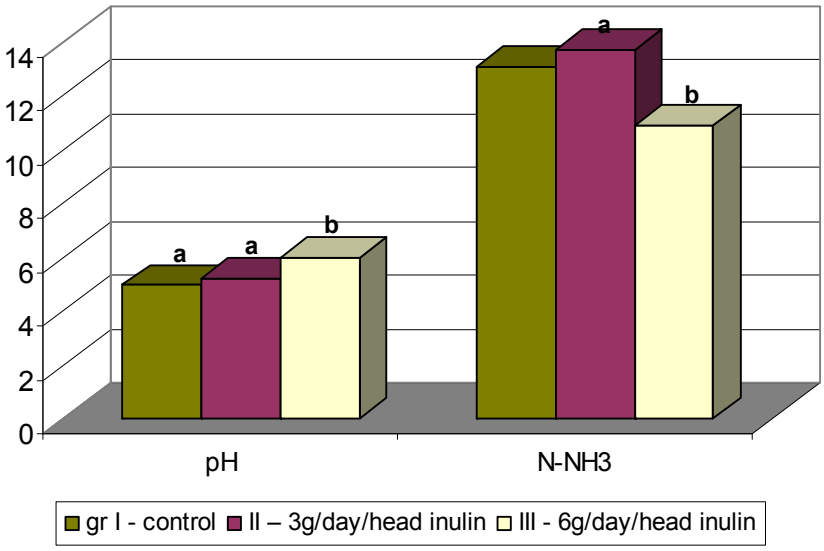

Fig. 9. Ammonium nitrogen [mg×dm^{-3}] and pH in calf rumen liquid (experiment II)

a, b – $P \leq 0.05$ – significant differences

Lower (P≤0.05) rumen pH was found in calves from control group compared to animals from experimental group that received inulin in amount 3 (gr II) i 6 g/day/head. (gr III). The highest ammonium nitrogen concentration in rumen fluid was stated in calves that received inulin in amount 3 g/day/head and it was clearly (P≤0.05) higher than in animals those milk replacers were supplemented by this prebiotic in amount 6 g/day/head (Fig. 9).

The highest (P≤0.01) volatile fatty acids concentration in rumen fluid was noted in calves that received milk replacer with inulin in amount 3 g/day/head (gr II). Whereas VFA concentration in rumen fluid of calves that received this oligosaccharide in amount 6 g/day/head was clearly (P≤0.01) higher than in control group and clearly (P≤0.01) lower than in animals that received inulin in amount 3 g/day/head (Fig. 10).

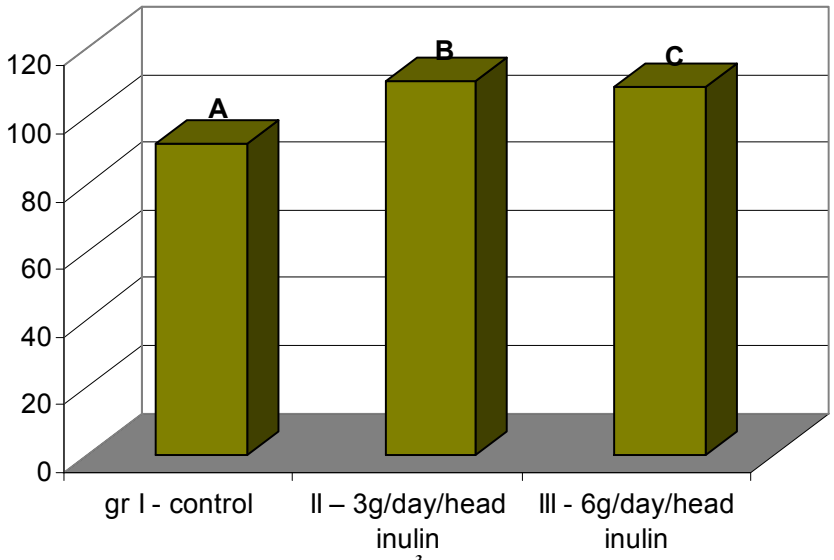

Fig. 10. Volatile fatty acids [mM×dm^{-3}] in calf rumen liquid (experiment II)

A, B, C – P ≤ 0.01 – high significant differences

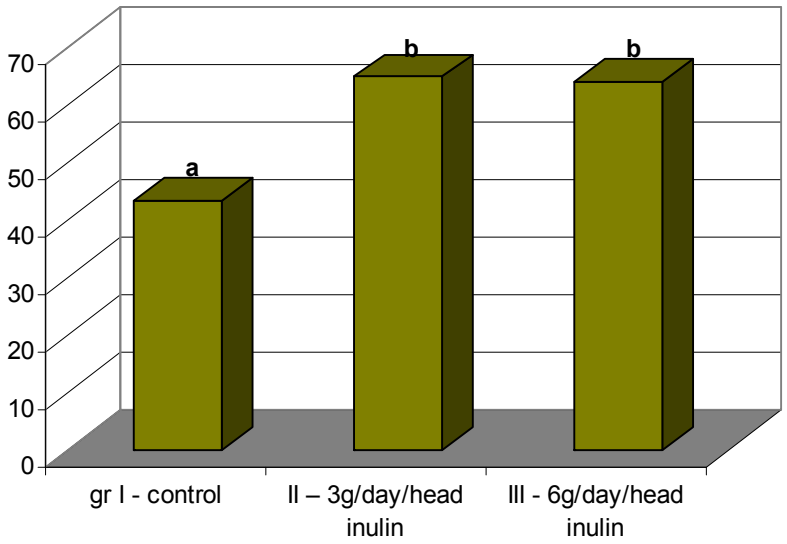

Fig. 11. Bacteria number [10^{9}] in calf rumen liquid (experiment II)

a, b – P ≤ 0.05 – significant differences

Applied in calf milk replacers inulin significantly effect on bacteria count in rumen fluid. Feeding calves with milk replacers containing inulin (gr II and III) affect increase of total bacteria count in 1 ml rumen fluid (P≤0.05) compared to control group (Fig. 11).

Applied in calf feeding inulin clearly effect on rumen protozoa count. Protozoa count was inversely proportional to rumen bacteria count. Number of rumen protozoa in calves that did not receive inulin was significantly higher (P≤0.01) than in calves that milk replacers were supplemented by this oligosaccharide. Calves that received inulin in amount 6 g/day/head showed higher (P≤0.01) protozoa count in rumen fluid than animals that received this prebiotics in amount 3 g/day/head (Fig. 12).

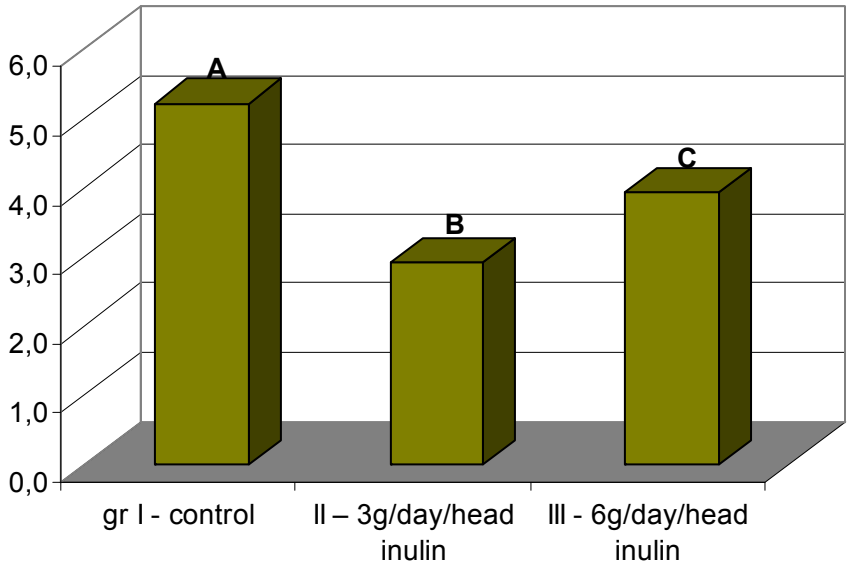

Fig. 12. Protozoa number [10^5] in calf rumen liquid (experiment II)

A, B, C – P ≤ 0.01 – high significant differences

Experiment III – yeast nucleotides

Initial body weight of calves was diversified and slightly higher in group II whereas final (56 day) body weight of calves that received yeast nucleotides (gr II and III) was higher than in animals from control group (gr I) (Table 6). Applied to calf milk replacers yeast nucleotides clearly(P≤0.01) increased daily body weight gains comared to control group. During all calf rearing (0–56 day) daily body weight gains in calves that did not received yeast nucleotides in milk replacer was significantly (P≤0.01) (P≤0.05) lower than in animals that milk replacers were supplemented by yeast nucleotides in amount both 2 and 4 g/day/head (Table 6). Calves that received yeast nucleotides in milk replacers (gr II and III) were shown higher concentrate mixture intake. Feed conversion (kg DM) per 1 kg body weight gains in animal receiving yeast nucleotides (gr II and III) was better than in control group. The best feed conversion was noted in calves that milk replacers were supplemented by yeast nucleotides in amount 4 g/day/ head and it was slightly (P≤0.05) differed from feed conversion in control group (gr I).

Table 6. Calf growth performance (experiment III)

Items	Feeding groups		
	I – control	II – 2g/day/head yeast nucleotides	III – 4g/day/head. yeast nucleotides
	$\bar{x}$ SD	$\bar{x}$ SD	$\bar{x}$ SD
Body weight:			
– after birth	43.57 ±5.01	46.20 ±5.14	42.72 ±6.07
– 56 day	71.61^{a} ±2.22	76.84^{b} ±2.08	77.55^{b} ±2.08
Average daily body weight gains [g/day]:			
	501^{Aa} ±81	547^{b} ±75	622^{B}±69
Concentrate mixtures intake [g DM/day]:			
	476^{a} ±23	530^{b} ±21	528^{b} ±47
Feed conversion [kg DM/kg body weight gains]:			
	3.33^{a} ±0.54	2.96 ±0.35	2.66^{b} ±0.29
Mortality[head]:	0	0	0

a, b – P ≤ 0.05 – significant differences, A, B – P≤ 0.01 – high significant differences

Fecal score in first rearing period (0–28 day) in calves that did not receive yeast nucleotides (gr I) was worse than in second rearing period (28–56 day). Given to calves yeast nucleotides in amount 2 and 4 g/day/head (gr II and III) improved fecal score those animal that was more equalized in both periods. Obtained data related to fecal score indicated that yeast nucleotides applied in milk repalcer reduced adverse influence of feed change as well as lower immunity and improved faeces consistency (Fig. 13).

Glucose level in calves from all feeding groups was similar. Calves that received yeast nucleotides in amount both 2 and 4 g/day/head (gr II and III) were demonstrated higher (P≤0.05) glucose content in blood than animals from control group. At the beginning of experiment level of serum urea in all animals was similar while in 56 day the highest (P≤0.05) urea level was stated in calves from control group (Table 7). Level of cholesterol and aspartate

aminotransferase in all calves did not differ from each other. At the end of experiment cholesterol level in animals that did not receive yeast nucleotides was clearly (P≤0.01) higher than in calves receiving this prebiotic in amount of 4 g/day/head or higher (P≤0.05) than in animals from group II (2 g/day/head). In 56 day aspartate aminotransferase level in calves from all groups was similar and it did not depend on applied to calf milk replacer yeast nucleotides (Table 7).

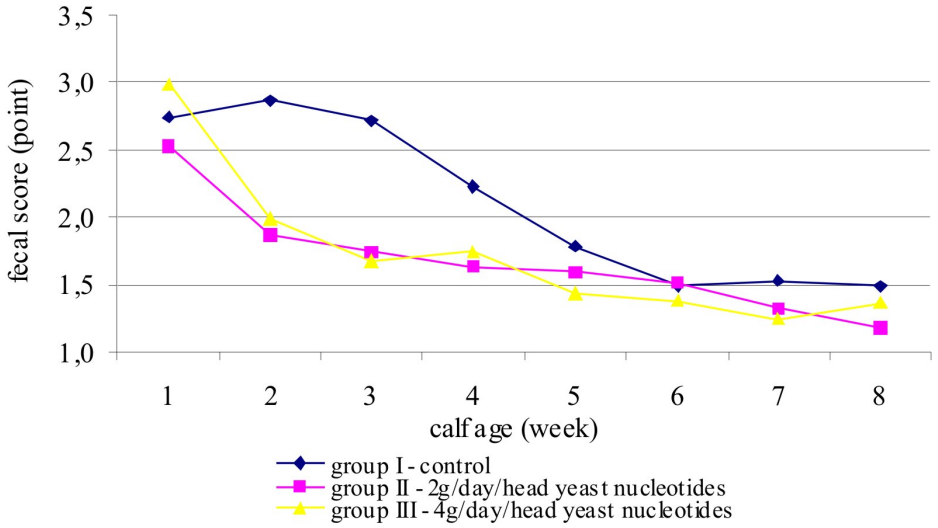

Fig. 13. Calf fecal score (experiment III)

Table 7. Biochemical parameters in serum and blood morphology (experiment III)

Items	Feeding groups		
	I – control	II – 2g/day/head yeast nucleotides	III – 4g/day/head yeast nucleotides
	$\bar{x}$ SD	$\bar{x}$ SD	$\bar{x}$ SD
Glucose [mg×dL^{-1}]:			
– 2–4 day	76.25±11.69	73.94±9.80	75.44±7.68
– 56 day	73.13±5.57	78.21^{a}±7.32	77.67^{b}±6.36
Urea [mmol×dm^{-3}]:			
– 2–4 day	3.60±1.86	3.89±1.07	4.30±1.05
– 56 day	4.05^{a}±0.63	3.42^{b}±0.39	3.26^{b}±0.45
Total cholesterol [mmol×dm^{-3}]:			
– 2–4 day	1.70±0.45	1.93±0.55	2.01±0.63
– 56. day	4.32^{Aa}±0.56.	3.01^{b}±0.32	2.22^{B}±0.35
AST [U×dm^{-3}]:			
– 2–4 day	64.30±27.97	54.40±20.22	79.80±29.30
– 56 day	57.86±30.87	69.83±14.12	56.60±20.73
White blood cells [10^{9}×dm^{-3}]:			
– 2–4 day	6.83±0.91	7.25±2.10	7.81±0.80
– 56 day	11.13^{a}±1.95	10.38±2.1	9.87^{b}±0.90
Red blood cells [10^{12}×dm^{-3}]:			
– 2–4 day	8.51±0.92	8.61±0.82	7.36±0.84
– 56 day	8.86±1.88	9.48^{a}±0.92	8.77^{b}±1.09
Platelets [10^{9}×dm^{-3}]:			
– 2–4 day	363±122	449±109	388±83
– 56 day	395±148	856±320	228±132
Hemoglobin [g×dL^{-1}]:			
– 2–4 day	8.90±0.88	7.92±1.15	8.13±1.17
– 56 day	8.81±1.26	9.54±1.05	9.97±1.04
Hematocrit [l×dm^{-3}]:			
– 2–4 day	0.292±0.04	0.268±0.04	0.261±0.04
– 56 day	0.224±0.07	0.325±0.05	0.294±0.06

a, b – P ≤ 0.05 – significant differences, A, B – P≤ 0.01 – high significant differences

In 2–4 day of life white and red blood cells count was similar in all calves. At the end of experiment white blood cells count in calves that received milk replacer with 4 g/day/head yeast nucleotides addition was lower (P≤0.05) than in animals from control group (gr I). In 56. day red blood cells count in calves that received this prebiotic in amount 2/day/head was higher (P≤0.05) than in animals from group III (4 g/day/head).The highest platelets number,

both in 2–4 and 56 day, was note in calves receiving yeast nucleotides in amount 2 g/day/head however those differences were not statistically confirmed (Table 7). Hemoglobin and hematocrit level in animals from all feeding groups both At the beginning and at the end of experiment was similar and it did not depend on yeast nucleotides given to calves in milk replacer.

In 2–4 day concentration of gamma-globulin in calf plasma in all feeding groups ranged from 8.19 – 13.93g×dm^{-3}. It means that calves could suffer from partial failure passive transfer (PFPT). Gamma globulin content in 21–28 and 56 day of life was slightly higher in calves that received yeast nucleotides than in animals from control group however by virtue of individual variability those differences were not statistically confirmed (Fig. 14).

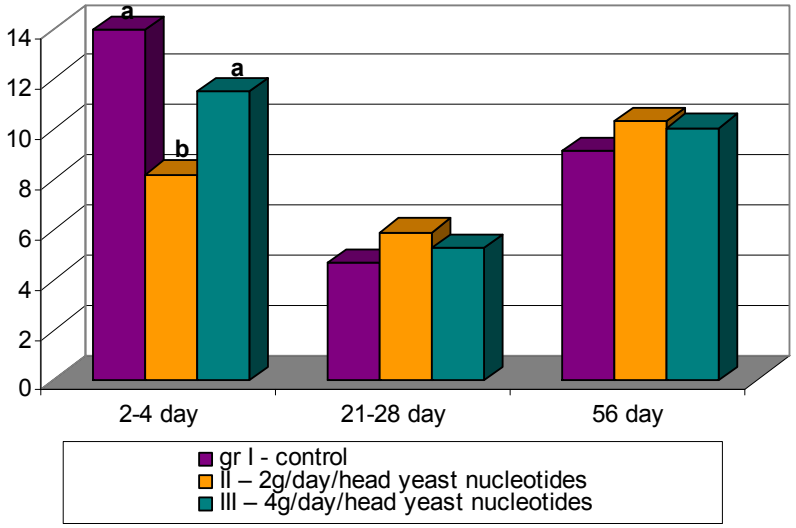

Fig. 14. Gamma-globulin concentration [g×dm^{-3}] in calf serum (experiment III)

a, b – P ≤ 0.05 – significant differences

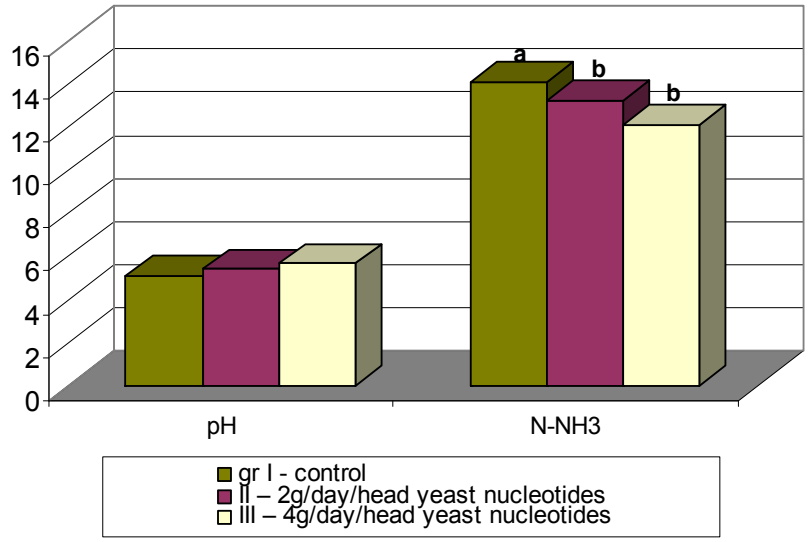

Fig. 15. Ammonium nitrogen [mg×dm^{-3}] and pH in calf rumen liquid (experiment III)

a, b – P ≤ 0.05 – significant differences

Higher rumen pH was noted in calves that received yeast nucleotides in milk replacer (gr II and III) than in animals from control group. Nitrogen ammonium in rumen fluid of calves from control group was higher (P≤0.05) than in animals from both experimental group (gr II and III) (Fig. 15).

Calves that were fed with milk repalcer containing yeast nucleotides in amount 4 g/day/head (gr III) demonstrated clearly higher (P≤0.01, P≤0.05) volatile fatty acids than animals from others groups (Fig. 16).

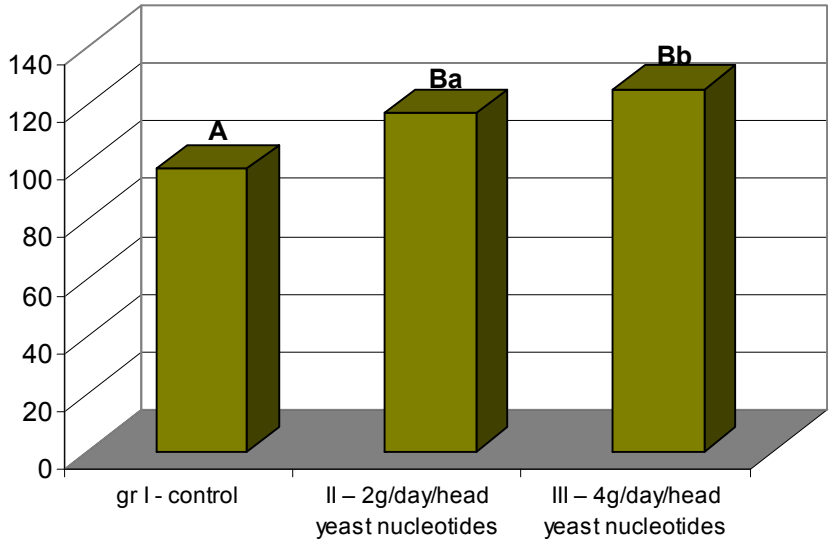

Fig. 16. Volatile fatty acids [mM×dm^{-3}] in calf rumen liquid (experiment III)

a, b – P ≤ 0.05 – significant differences**,** A, B – P ≤ 0.01 – high significant differences

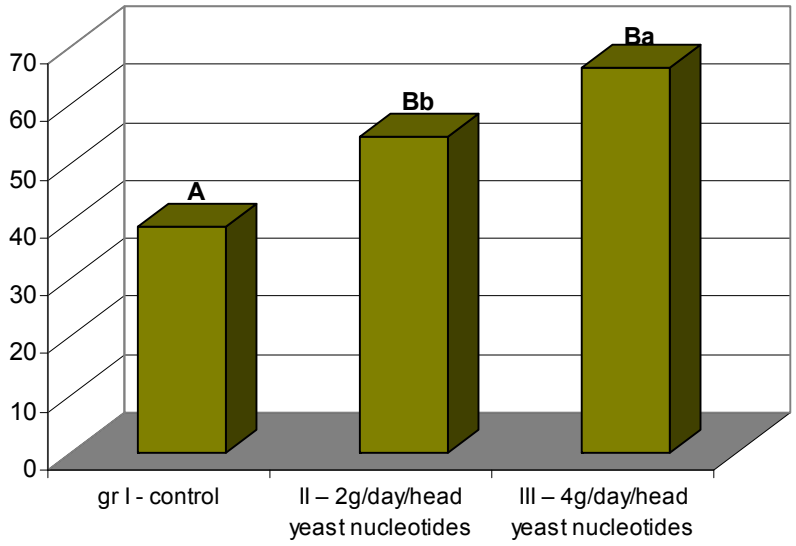

Fig. 17. Bacteria number [10^{9}] in calf rumen liquid (experiment III)

a, b – P ≤ 0.05 – significant differences, A B – P ≤ 0.01 – high significant differences

Applied in calf milk replacers yeast nucleotides enlarged number of bacteria in rumen fluid. Given to calves yeast nucleotides in amount both 2 and 4 g/day/head (gr II and III) clearly increased total bacteria count compared to control group (gr I). Bacteria number in rumen fluid of calves that fed milk repalcer with yeast nucleotides in amount 2 g/day/head was lower (P≤0.05) than in animals that received this additive in amount 4 g/day/head (gr III) (Fig. 17).

Given to calves in milk replacers yeast nucleotides significantly influence on protozoa count in rumen fluid. Number of rumen protozoa was inversely proportional to rumen bacteria number. The lowest total number of protozoa was noted in calves that received yeast nucleotides in amount 4 g/day/head and it was clearly lower (P≤0.01) than in animals from others groups (Fig. 18).

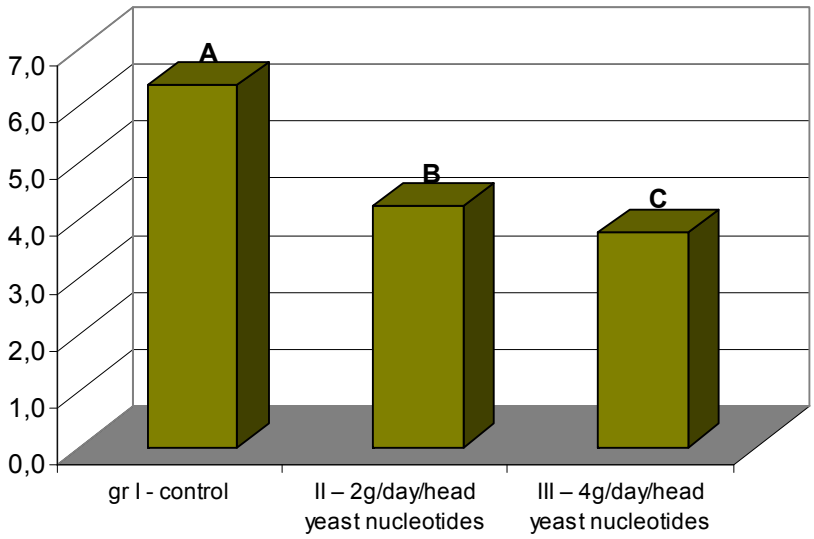

Fig. 18. Protozoa number [10^5] in calf rumen liquid (experiment III)

A, B, C – $P \leq 0.01$ – high significant differences

DISCUSSION

Obtained data related to calf rearing show that additives applied in milk replacer, especially mannanooligosaccharides (MOS) and yeast nucleotides affected higher body weight of calves, better concentrate mixture intake and feed conversion. Also Newman [33] in researches on Holstein breed calves noted that given in milk replacer mannan oligosaccharides in amount 2 g/day/head increased daily body weight gains. Higher body weight gains, clearly ($P \leq 0.01$) higher concentrate mixture intake and better feed conversion were stated by Lubojemska and Kinal [30] in experiment carried out on newborn calves that fed milk replacer with MOS in amount 2 and 4 g/day/head. On the other hand Heinrichs et al. [20] showed that added to calf milk replacer antibiotic (400 g/tonne of neomycin and 200 g/tonne of oxytetracyclin) and mannanooligosaccharide (4 g/day/head) did not affect daily body weight gains whereas concentrate mixture intake was higher ($P \leq 0.05$) than in calves receiving antibiotics. Kaufhold et al. [25] demonstrated that inulin and fructooligosaccharides applied to calf milk repalcers did not effect on feed intake but improved feed conversion what was strictly connected with improvement of digestion processes and more effective nutrients utilization.

According to Milewski et al. [31] and Suzuki et al. [40] glucans can stimulate body weight gains and traits of meat utility in animals.

The results of calf fecal score showed that examined prebiotics, especially MOS and yeast nucleotides improved fecal score what could confirm their better health condition. Also Lubojemska and Kinal [30] in studies on newborn calves that received 4 g/day/head MOS in milk replacer stated improvement of fecal score. As Newman [33] wrote MOS administered to calves in amount 2 g/day/head slightly decreased *E.coli* count in faeces in 3–4 week of their life while in 5 week *E.coli* count was significantly lower. Badyoczek [2] in *in vitro* study on isolated intestine flora demonstrated that MOS is able to absorb and inhibit proliferation of pathogen strains – proliferation of *E.coli, Salmonella* and *Clostridium* was limiting at the same time proper conditions were ensured to proliferation of *Bifidobacteriae* and *Lactobacillus*. Better fecal score, less severe diarrhea and shorter their lifetime were noted by Quigley [36] in researches on calves that were fed with milk replacers containing fructose oligosaccharides.

Bunce et al. [7] proved that fructooligosaccharides administered to calves affected *Bifidobacteriae* proliferation in faeces whereas *Escherichia coli* count was decreased. According to Kulkarni et al. [28] and Yamamoto et al. [45] there is relation between nucleotides content in diet and increase resistance on many potential pathogens what ca lead to decrease occurrence and severity some bacterial and fungal diseases.

Prebiotics applied in milk repalcers mannanooligosaccharides, inulin and yeast nucleotides did not clearly effect on marked biochemical and morphological parameters in blood and serum. The glucose content in blood of calves that received mannanooligosaccharides, inulin and yeast nucleotides was higher than in animals from control group. It was probably an effect of higher energy intake and better its absorption and utilization in tissues.

Increase of glucose content in blood of young calves is strictly connected with higher body weight gains. Skórko-Sajko and Sajko [38] did not find any MOS effect on glucose and urea level in experiment carried out on calves that received mannanooligosaccharides in amount 4 g/day/head. Similarly Lubojemska and Kinal [30] did not stated

influence of this prebiotic on glucose content in blood. Also Quigley et al. [36] did not note clearly differences in glucose content in Jersey breed calves that received fodder yeast during first 12 weeks of life. Whereas Dobicki et al. [14] found that 4% dried brewer's yeast addition to concentrate mixture effected on lower ($P \leq 0.05$)glucose content than in calves that did not receive this prebiotic. The examined prebiotics added to calf milk replacer decreased serum urea content compared to calves that did not receive this prebiotics. It could be an effect of rumen fermentation change – increase of protein synthesis by rumen microorganisms leads to serum urea content decrease [6]. Zornborszky-Kovacs et al. [48] found lower urea level in sheep in that received termolysed yeast. Obtained in own researches data related to cholesterol level in calf serum showed that applied additives, especially inulin clearly decreased total cholesterol. It is connected with inulin capacity of HMG-CoA activity inhibition what consequently affect HMG-CoA concentration – key semi-product of cholesterol.

Applied to milk repalcers mannanooligosaccharides, inulin and yeast nucleotides did not affect blood morphology and all stated differences were results of health state and calves age. According to Knowles [26] red blood cells count in newborn calves should range from 8.5 to $10.5 \times 10^{12} \times dm^{-3}$. Davis et al. [12] wrote that mannans added in weaning piglets nutrition caused normalization neutrophiles count and increased lymphocytes count. It pointed to fact that mannans enriched diet had beneficial influence on immunity system and co-operate in homeostasis keeping in animal with inflammatory process. According to Kulkarni et al. [28] normal functioning of bone marrow, red and white blood cells id depended on nucleotides content in diet. In experiment conducted on pregnant ewes Zomborszky-Kovacs et al. [48] found that yeast nucleotides given to pregnant ewes and Suffolk lambs in amount 1.5 g/day/head affected increase haematopoesis' process and proliferation of red and white blood cells.

The results of serum analyses taken from calves after finished immunity passive transfer proved that calves from all group were relatively good supplied with colostrum immunoglobulin. Average immunoglobulin level was $11.12 g \times dm^{-3}$ what should be recognized as failure passive transfer [16]. It is often assumed that immunoglobulin concentration in calf serum lower than 10 $g \times dm^{-3}$ is equivalent to failure passive transfer [8,41]. The papers that have distinguished partial failure passive transfer are barely met [5,16]. It seems to be wrong because those calves should have been additional protected. It is well-known that adequate passive immunity is essential for health and development not only in first weeks of their life but can also effect on their later performance. As well protected are regarded calves that serum immunoglobulin concentration is higher than $15 g \times dm^{-3}$ [37]. According to Holloway et al. [22] and Quigley et al. [36] proper level of serum immunoglobulin in second day of life should amount more than $13 g \times dm^{-3}$. In own researches the highest serum immunoglobulin concentration was found in experiment I while the worst values were noted in experiment II. However average serum immunoglobulin concentration in all calves could be regarded as sufficient provide that proper zoohygienic conditions will be ensured.

The lower pH and nitrogen ammonium concentration as well as the higher volatile fatty acids concentration in rumen fluid were fund in calves that received prebiotics in milk replacers. According to Koenig et al. [27] rumen pH increase (more unlikely acidosis occurrence) could be explained by partial defaunacy of rumen ecosystem as a result of brewer's yeast addition. Those authors wrote that lower nitrogen ammonium in rumen fluid is observed in defaunated animals. As Dawson stated [13] specific yeast product Yea-Sacc^{1026} increased microbiologic activity of rumen via nitrogen metabolism change in rumen (reduction of ammonium concentration). Therefore obtained in own researches data related to reduction of ammonium nitrogen concentration could be a result of rumen protozoa number decreased in calves that received those prebiotics. According to Nisbet and Martin [34] as well as Chaucheyras [10] hydrolysed yeast as a source of nutrients, vitamins, especially from B group, polyphenols and organic acids stimulate proliferation of rumen bacteria. Dobicki et al. [15] found increase (34–39%) of bacteria count in rumen fluid was noted in milk cows that received brewer's yeast in amount 80 g/day/head and mannan oligosaccharides in amount 20 g/day/head during first 100 day of lactation. After those Authors it could affirm better utlilization of nutrients, especially fiber from ration. Also Newbold et al. [32] in study carried out on sheep that received *Saccharomyces cerevisiae* yeast noted increase (38%) bacteria count in rumen fluid samples. The prebiotics (mannanooligosaccharides, inulin and yeast nucleotides) applied to milk replacers clearly decreased total protozoa count in rumen fluid. Dobicki et al. [14] noted similar results in calves that received dried brewer's yeast in concentrate mixture. As Wojciechowicz [44] wrote a lower ammonium concentration in rumen fluid is observed in partly defaunated animals. It could indicated better ammonia utilization by bacteria. Ivan et al. [23] and Whitelaw et al. [42] claimed that lack of rumen protozoa stimulate volatile fatty acids production, especially propionate. It was also confirmed in own researches.

CONCLUSIONS

The results of research related to effect of mannanooligosaccharides, inulin and yeast nucleotides added to calf milk replacers on calf rearing and health condition affected:

- higher final body weight as well as higher daily body weight gains;
- better concentrate Prof. Dcsintake and feed conversion ratio all rearing long;
- better faeces scores – better consistency (less watery and well formed);
- higher glucose content increased while levels of cholesterol and plasma urea N were lower;

- higher level gamma-globulin as well as better passive immunity transfer were stated in calves receiving mannanooligosaccharides and yeast nucleotides in amount 4 g/day/head in milk replacer;
- higher calf rumen pH values and lower level of rumen ammonium nitrogen;
- higher oncentration of total volatile fatty acids in rumen,
- increase in the total bacteria count and decrease in the concentration of protozoa in the rumen fluid.

Results obtained in the experiments indicate that addition of prebiotic feed additives to calf milk repacers, particularly mannanooligosaccharides and yeast nucleotides, is recommending.

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DSc. Andrzej Zachwieja, associate professor

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