INFLUENCE OF VARIOUS CONCENTRATIONS OF SYNBIOTIC FEEDING ON THE GROWTH AND DEVELOPMENT OF CALVES DURING THE FIRST FOUR MONTHS OF POSTNATAL DEVELOPMENT

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Abstract

*The use of antibiotics for growth promotion and for disease prevention in production animals has been banned in countries of European Union since the 1st of January, year 2006. Alternative feed additives were needed to achieve the same or even better results in animal husbandry. As one of the possible antibiotic alternatives, prebiotics are the one that can prevent diseases and provide good health. We know that prebiotics, probiotics and synbiotics have a positive effect on monogastric animal organisms, but their effects on multi-chamber stomach animals have not been sufficiently studied. The studies were carried out to determine the effect of feed on calves at the age of 4-12 weeks, feeding the flour concentrate of Jerusalem artichoke which was produced in Latvia (contains prebiotics - inulin 48.5% - 50.1%) and probiotic Enterococcus faecium (1 g (2 * 10^9 CFU / g) that were added to milk. The study was carried out on 40 calves of average age 23 ± 5 days. Three doses of synbiotics were tested. Once a day was performed a general health check of each calf, with special emphasis on fecal mass consistency and body measurements were performed once a week. The study lasted for 56 days. We found that the calves of the synbiotic group had less cases of alimentary diarrhea than animals of the control group and the average body weight gain in the synbiotic group was significantly higher ($p < 0.05$) than in the control group. The study was carried out within the AGROBIORES State Research Program.*

Key words: *calf, inulin, Enterococcus faecium, synbiotics*

1. INTRODUCTION

Since the beginning of 2006, the use of antibiotics in animal nutrition to promote the growth of animal live weight has been prohibited in the European Union. There are no longer doubts that the use of antibiotics affect both the unfavorable and the beneficial microflora but does not strengthen or enhance the animal's immune system activity. The fight against antibiotic-resistant microorganisms makes this problem even more urgent. Therefore, alternative means are being sought to accelerate live weight gain, improve general health condition and reduce mortality without harming both animal and human health (Mathur et al. 2005; Verdonk, 2005; Heinrichs et al. 2009). Studies on the influence of prebiotics, probiotics and synbiosis on monogastric animals and the human digestive system, health and the immune system show that they are the successful antibiotic alternatives (Samanta 2013, Masanetz et al. 2010).

It should be acknowledged that the morpho-functional characteristics of the ruminant animal's digestive system do not allow the unequivocal use of the same prebiotics, probiotics or synbiosis that are used for monogastric animals, as their relatively delicate paunch ecosystem can be damaged, and the desired effect will not be achieved. In addition, synbiotics or its components that exert a beneficial effect on the intestinal activity of monogastric animals may not reach intestinal system of ruminant species.

Our previous studies have shown that prebiotic inulin stimulates the growth of multichambered stomach in ruminant animals and improves the health condition of calves in general (Arne & Ilgaza 2016; Ilgaza, Arne et al. 2016; Jonova, Ilgaza & Grinfelde 2017), but the combination of inulin with some probiotics - thus, synbiotics - is relatively small. One of the most studied probiotics for ruminants is *Enterococcus spp*. bacteria (Nocek et al. 2002; Corcionivoschi et al. 2010; Uyeno et al. 2015). *Enterococcus faecium* is a lactic acid bacterium that is a normally found in the gut and it shows effects against enteropathogens

(Willard et al. 2000; Benyacoub et al. 2005).The probiotic *Enterococcus faecium* initiates a positive feedback mechanism to stimulate the metabolic activity of lactobacilli (Vahjen et al. 2002).

In our study, we would like to supplement our already undertaken research on influence of the prebiotic inulin as a potential component of the synbiotic, which contained in the Jerusalem artichoke flour produced in Latvia, on the health status and condition of calves. Generally, Jerusalem artichoke flour contains an average10% of inulin, but specially designed technology allows to increase the amount of inulin up to 48.5% - 50.1% (Fleming et al. 1979; Valdovska et al. 2012). This facilitates to add inulin to edible fodder, in our study, milk. Therefore, the aim of our study was to find out the feeding effect of a new symbiotic product which consists of an increased concentrate of inulin *Helianthus tuberosus* (inulin 48.5 - 50.1%) flour produced in Latvia and industrially produced *Enterococcus faecium* on calf's general health and digestive tract health condition and increase in live weight.

2. MATERIALS AND METHODS

2.1. Materials

For the study we took Holstein Friesian crossbreed male calves of 4 weeks of age, kept and fed under the same conditions. All calves before and after the start of the study had the same husbandry and feeding conditions that met the prescribed species and age requirements. The types of the feed and nutrients can be seen in table 1. At the beginning of the study, we carried out a general clinical health check for all calves, which included the counting of heart rate, respiratory rate, measuring the body temperature and evaluating the faecal consistency. In the study we included only clinically healthy 23 ± 5 day old calves with a live weight of 51 ± 2 kg.

Item	Unit	Prestarter*	Milk replacer**	Hay	Barley flour	Forage
Crude protein	%	18.0	23.5	9.3	14.3	3.8
Fat	%	5.2	12.5	2.4	2.7	1.1
Crude fiber	%	7.0	3.5	32.1	3.3	10.0
Crude ash	%	6.5	9.0	5.6	4.4	2.4
Calcium	g/kg	14.0	9.0	3.3	4.4	1.5
Phosphorus	g/kg	7.0	7.0	3.0	4.0	1.0
Sodium	g/kg	6.0	4.0	0.2	16.2	0.1

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

***** *Composition per kilogram of diet: vitamin A, 35.000 IU; vitamin D3, 3.000 IU; vitamin E, 250 mg; vitamin B1, 13 mg; vitamin B2, 6 mg; vitamin B6, 5 mg; vitamin B12, 40 mcg, niacin, 36 mg; Ca-D-pantothenate, 20 mg; folic acid, 2 mg; vitamin K3, 2 mg; choline chloride, 500 mg; biotin 1.000 mcg; Fe, 180 mg; Cu, 25 mg; Se, 0.4 mg; Zn, 120 mg; Mn, 120 mg, I, 3 mg; Co, 1 mg*

****** *Composition per kilogram of diet: vitamin A, 48.000 IU; vitamin D3, 4.000 IU; vitamin E, 100 mg; vitamin B1, 16 mg; vitamin B2, 8 mg; vitamin B6, 6 mg; vitamin B12, 50 mcg, niacin, 50 mg; vitamin C, 250 mg; Ca-D- pantothenate, 25 mg; folic acid, 1 mg; vitamin K3, 2 mg; choline chloride, 300 mg; biotin 200 mcg; Fe, 100 mg; Zn, 36 mg; Cu, 4 mg; Se, 0.4 mg; Mn, 48 mg, I, 1 mg*

2.2. Methods

Animals were divided into four groups, each group had 10 animals. At the starting day of the study, control calves (Sim0, n = 10) received milk 3.5 liters twice per day, water and hay were available without restriction. In the three study groups, calves were additionally fed with 1 g of *Enterococcus faecium* (1

g (2 * 10^9 CFU / g) and a corresponding quantity of Jerusalem artichoke flour concentrate: each calf of the Sim1 group (n = 10) received 6 g of Jerusalem artichoke flour (i.e. 3 g inulin), each calf of the Sim2 group (n = 10) 12 g (6 g inulin) and each calf of Sim3 group received 24 g of flour of Jerusalem artichoke (12 g inulin) per day. The study lasted for 8 weeks (56 days) (a similar scheme in the study is described by Krol 2011. Each group was kept in a separate pen; they had free access to drinking water and hay all days' round, as well as forage for two weeks after the start of the study.

During the study, we evaluated the health status of calves each day paying a special attention to the faecal consistency. Animal faeces were evaluated each day, where 0 point was for solid faeces, meaning no diarrhea signs, but 3 points for watery faeces (Larson et al. 1977). During the control weighting a very attentive general health check (we measured respiratory and heart rate, as well as measured the body temperature) also was carried out. Upon reaching 12 weeks of age, a planned slaughter of calves in a certified slaughterhouse was carried out. After slaughter, we made morphometric examination and measurement of the digestive tract.

Starting the study and once a week during the study, the animals were checked for weight. MS Excel and the R-Studio were used for data processing. Values of physiological and weight gain parameters are given as mean ± standard deviation (SD). Significance was tested by applying Student's t-test. Values of less than 0.05 ($p < 0.05$) were considered significant.

3. RESULTS AND DISCUSSION

One of the research tasks was to ascertain how the feeding of synbiotics affects the general health status of calves and whether the fed dose of synbiotics had influence on that. It should be noted at once that none of the animals showed serious health problems during the study. However, to clear up whether these indices differed among the groups, we calculated the mean and scattering rates of the heart rate, respiratory rate and body temperature in each group at each general health check. In the calculation, we found that there were no statistically significant differences in these measurements among the different groups of animals ($p > 0.05$) and the indicators of all groups were within the normal range, as shown in the Table 2.

Group	Heart rate x/min ± SD	Respiratory rate x/min ± SD	T° ± SD
Sim0	124.0±16.76	31.3±2.19	39.20±0.87
Sim1	112.8±10.73	32.0±2.83	39.18±0.48
Sim2	108±12.00	31.2±3.35	39.02±0.62
Sim 3	108±12.00	31.2±3.35	39.04±0.56
The normal range (Mohra et al., 2002)	86-125	15-40	38.0-39.5

Table 2. Physiological indicators for 4-12 weeks old calves with different doses of synbiotic

We were particularly interested in the influence the synbiotics on the digestive tract's health status. Every day, we performed a visual evaluation of fecal mass consistency in points from 0-3 (Larson et al. 1977). It should be noted that heavy, watery diarrhea (evaluated in 3 points) was not observed during the study. To make the results more visible, we compiled the results by calculating the average point for each group of animals per week. At the start of the study, the average values for all groups of animals did not exceed the 0.5-point limit - so no diarrhea was detected in animals. This situation lasted for one more week (Figure 1).

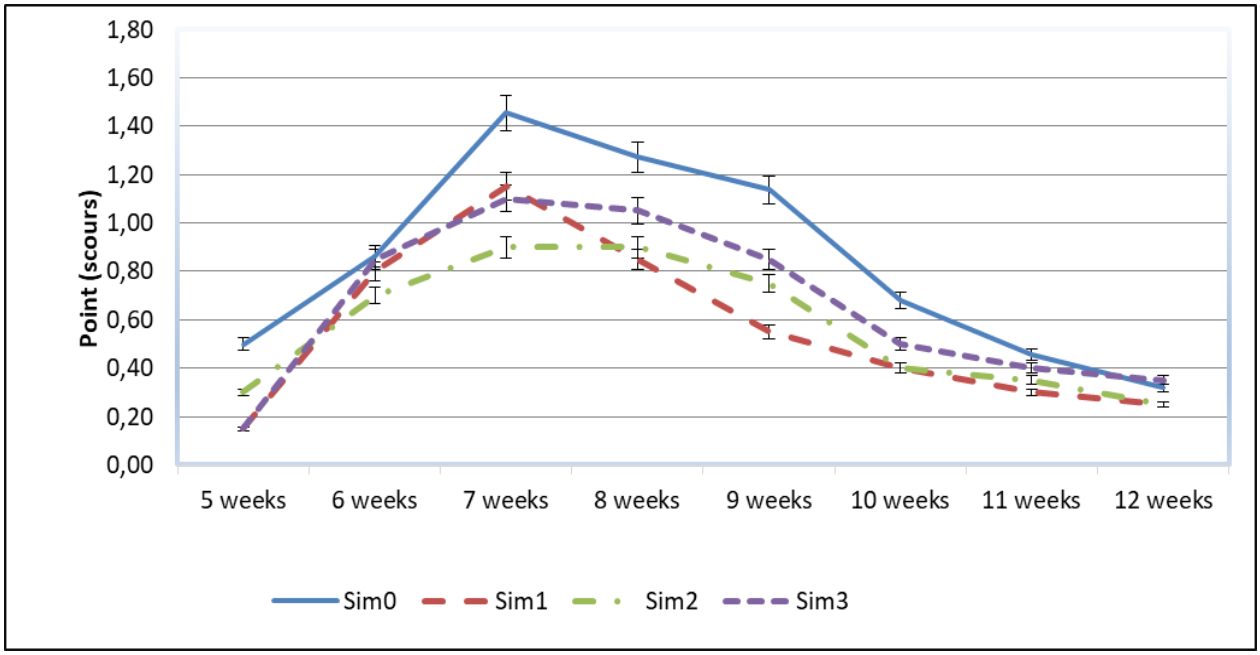

Fig. 1. Evaluation of faecal consistency of calves (5-12 weeks old) not feeding (Sim0) or feeding different doses of synbiotics (Sim1, Sim2, Sim3 groups)
(Calculating values in 0-3 points by Larson, 1977).

We observed significant liquefying of fecal mass in 7-week-old calves. The worst indicators turned out to be in control group of animals (1.45 ± 0.6 points), it had been the worst indicator throughout the study. The Sim2 group had a relatively better situation (0.7 ± 0.35) that week. It should be noted that two weeks after the start of the study, calves were offered fodder that could be the cause of changes in fecal mass consistency - alimentary diarrhea. In the further study comparing the results obtained, the fecal mass consistency of animals in the control group was more watery than normal for up to 10 weeks of life, while in synbiotic groups Sim1 and Sim2 it dropped below 1 point already on the at 8th week, in Sim3- on the 9th week. On the twelfth week of life, the fecal mass consistency of all groups of animals was close to 0 and diarrhea was not observed in any animal in any of the study groups. We observed that on the 11-12th week of life, animals began to eat intensively coarse food or hay, due to that it could be possible the fecal mass became tighter and no diarrhea was observed. It also can be due to the fact that during the first 12 weeks of life, the most intense bacterial growth of the intestinal tract occurs (Yutaka et al. 2015), which provides stabilization of the intestinal tract.

In general, our results coincide with the study described by Samanta and co-authors (2011), where prebiotics were fed to calves, as they also found that the fecal mass consistency under the influence of prebiotics was more stable and better. There are also many other studies which have revealed that adding the prebiotic inulin to the milk replacer of pre-ruminant calves leads not just to significantly higher weight gains but also this additive significantly improves faecal consistency (Kaufhold, Hammon & Blum 2000; Verdonk & Van Leeuwen 2004).

The third table shows the live weight (average indices and standard deviation) of Sim0 and Sim1, Sim2, Sim3 groups every two weeks, as well as the average live weight gain comparing day 1 and day 56 with average live weight gain throughout the study.

Groups	Average body weight (kg/the research day)			Average daily body weight gain
	First day	**28th day**	**56th day**	
Sim0	50.8±1.25	69.1±5.97	82.2±6.53	0.3±0.13
Sim1	51.0±1.25	75.4±4.35	100.1±3.14	0.8±0.06
Sim2	51.7±1.06	77.4±1.65	103.0±2.26	0.9±0.09
Sim3	52.1±1.73	74.6±1.19	99.8±3.36	0.8±0.09

Table 3. Average weight of the calf (5-12 weeks old) at the research day not feeding (Sim0) or feeding different doses of synbiotics (Sim1, Sim2, Sim3 groups)

We found that throughout the study period, synbiotic animal groups had a more stable increase in live weight than control group of calves. Already on the 28th day of study (at age of 8 weeks), the live weight of the synbiotic group of calves exceeded the live weight of the control group animals more than by 5 kg. On the 56th day of the study, we found a statistically significant difference between all synbiotic groups and the control group ($p < 0.05$), where the weight of the control group 82.2 ± 6.53 kg was statistically significantly lower. It should be noted that Sim2 group animal live weight (103.0 ± 2.26 kg) and average daily body weight gain (0.9 ± 0.09) were statistically significantly higher in comparison to Sim1 and Sim3 groups ($p < 0.05$), which indicates, that when feeding the average dose of inulin, its impact is most effective. Similar, but not so high, results were reported by other authors in their studies (Stolic et al. 2011; Roodposhti & Dabiri 2012). It should be noted that too big synbiotic feed dose does not improve live weight gain, because the Sim3 group received 12 grams of inulin and 1 g of *Enterococcus faecium* provided a significantly higher result (99.8 ± 3.36 kg, 0.8 ± 0.09 kg) than in the control group (82.2 ± 6.53; 0.3 ± 0.13), but it was lower among synbiotic groups.

In many studies, authors Mohmadi (2011), Roodposhti & Dabiri (2012) and Marcondes et al. (2016) proved that calves which were fed a variety of synbiotics, had the highest weight gain and food intake compared with the animal control group. Simon and co-author (2001), described in his study that when probiotic *Enterococcus faecium* was added to feed, significant weight gain was not achieved compared to the control animals. Our study proves that, with the addition of inulin and bacteria to feed, the increase in live weight in all synbiotic groups is significantly higher than in the control group. It is possible that the addition of inulin to this probiotic improves its performance; therefore, the research in this direction still needs to be continued.

4. CONCLUSIONS

Feeding synbiotics (inulin and *Enterococcus faecium)* to 4-12-week-old calves does not significantly affect calves' heart rate, respiratory rate and body temperature and remain within the limits of the physiologically normal range. The optimal dose of inulin is synbiotic with 1 g of *Enterococcus faecium* (1 g ($2 * 10^9$ CFU / g) of 12 g per animal per day with milk. There were less cases of alimentary diarrhea in this group, which resulted in higher and more stable live weight gain per day and significantly higher live weight gain during the 56 study days.

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