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The effect of synbiotic inulin and enterococcus bacteria on digestive health and weight gain in calves

A. Arne* and A. Ilgaza

Latvia University of Life Sciences and Technologies, Faculty of Veterinary Medicine, K. Helmaņa street 8, LV-3004 Jelgava, Latvia
*Correspondence: arne.astra@gmail.com

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Abstract. The aim of study was to investigate the effect of a synbiotic containing *Enterococcus* bacteria and 3 different concentrations of inulin on the performance and health status of calves. Forty randomly selected healthy male Holstein crossbreed calves 23 (+/- 5) days old and weighing 50 kg (+/- 5 kg) were randomly allocated to 4 groups: control group (CoG $n = 10$) fed only whole milk, and 3 synbiotic supplemented groups: 1) SynG6 $n = 10$, 2) SynG12 $n = 10$, 3) SynG24 $n = 10$, in which calves received various amounts of prebiotic inulin (artichoke powder 6 g, 12 g, and 24 g) with 0.25 g of the probiotic *Enterococcus faecium* ($2{\times}10^9$ CFU g^{-1}). At the end of this study all three synbiotic group weight gains were significantly greater than the control group ($p < 0.01$). SynG12 (12 g artichoke powder) group's weight gain was significantly greater than control and the 6g and 24 g synbiotic groups ($p < 0.05$). The average cold carcass weight results were similar to the live weight results: SynG12 was significantly ($p < 0.05$) higher than SynG6 and SynG24. Supplementing feedings with this combination of the synbiotic containing 6 g of inulin (produce in Latvia) mixed with *Enterococcus* (Protexin, UK) bacteria (SynG12) was most effective in achieving the greatest daily weight gain and cold carcass weight.

Key words: calf, *Enterococcus faecium,* feeding, inulin, synbiotic, weight gain.

INTRODUCTION

Carcass quality in ruminants is determined more by dietary components than by the general health status (Blanco-Penedo et al., 2009). Antibiotics are frequently used to achieve this goal. Thus, it is important to identify new and better alternatives to antibiotic use for increasing animal weight gain and general animal health improvement. Probiotics, prebiotics and various combinations of these substances called synbiotics have been studied (Masanetz et al., 2011; Samanta et al., 2013). One of the most researched probiotics for ruminants is *Enterococcus spp*. bacteria (Nocek et al., 2002; Corcionivoschi et al., 2010; Uyeno et al., 2015).

Prebiotics are not digested in the gastrointestinal tract but rather they support the growth and development of beneficial intestinal microflora which limits plaque formation by pathogenic microorganisms such as *Salmonella sp.* or *Escherichia coli* improving the overall health of the animal (Gibson & Roberfroid, 1995; Patel & Goyal, 2012; Kara et al., 2015).

The synbiotic term is used when 2 different substances are combined such as probiotics with prebiotics in order to improve function, digestibility, motility and general health of the intestinal tract and the animal (Uyeno et al., 2015; Markowiak & Śliżewska, 2018). Several studies have shown that inulin, derived from Jerusalem artichoke, improves the health and growth of non-ruminants and chickens (Velasco et al., 2010; Valdovska et al., 2014; Samolińska et al., 2018), but there is no data on feeding synbiotics to ruminants.

In this study, the synbiotic - inulin from artichoke flour (inulin 48.5–50.1%) combined with *Enterococcus faecium* (2×10^9 CFU g^{-1}) was selected in order to test if this supplement could significantly improve calf health by increasing the functionality of the digestive system and thus, enhancing animal live weight gain.

MATERIALS AND METHODS

Study Herd, Housing and Feeding

The research was carried out on a 420 cow dairy farm in Latvia. Forty randomly selected, 23 (± 5) days old healthy male Holstein crossbreed calves, weighing 50 kg (± 5 kg), were randomly allocated to 4 groups. The study started when the animals had reached 4 weeks of age. The calves were kept in groups of 10, under the same conditions and were fed twice a day, ~3.5 liter of whole milk per feeding. The pen for 10 calves was 25 m^2.

Water and hay were freely available 24 hours per day, and fodder was added two weeks after the start of the study when the animals were 6 weeks old. Composition of the concentrated feed and Jerusalem artichokes flour composition is shown in Table 1.

Table 1. Composition of Concentrated Feed and Jerusalem Artichokes Flour for Study Animals

	Composition (g kg^{-1} Dry Matter basis)					Composition (g mg^{-1} Dry Matter basis)				
	dry matter	CP	NDF	ADF	starch	inulin	free glucose	free fructose	sacc-harose	nucleic acids
Concentrated feed	882	142	481	34	655	-	-	-	-	-
Artichoke powder	950	171	-	-	635	495	8	26	106	21

CP – Crude Protein; NDF – Neutral Detergent Fibre; ADF – Acid Detergent Fibre.

The calves were divided into 4 groups: control group (CoG, *n* = 10) was fed only whole milk, and 3 different synbiotic amount groups: SynG6, *n* = 10, SynG12, *n* = 10, SynG24, *n* = 10, in which the calves received 3 different amounts of the prebiotic inulin mixed with 0.25 g of the probiotic *Enterococcus faecium* (2×10^9 CFU g^{-1} (Protexin International Ltd., South Petherton, UK)). Jerusalem artichoke powder and Enterococcus faecium were added to the first one of the two daily milk feedings. In this research, the prebiotic we used was artichoke flour concentrate, produced in Latvia at the University of Latvia Institute of Microbiology and Biotechnology, the inulin was 50% DM, thus, each 12 g of artichoke powder contained approximately ~6 g of inulin.

Health monitoring

Throughout the study, daily calf health was evaluated by close visual inspection of the fecal consistency. Animal faeces were evaluated in points, where 0 - points score was for solid faeces without diarrhea sign, 1 - point was for soft faeces with maintained consistency, 2 - points were for liquid feces with lost solidity, and 3 points for watery faeces (Larson et al., 1977).

The 4 week old calves were weighed at the beginning of the study and every two weeks during the study (6, 8, 10, 12 weeks). During the control weighing, a general health exam was done and the main physiological indicators were recorded.

The study lasted 8 weeks or 56 days. A similar study framework has been used by other authors (Król, 2011). The planned slaughter of calves occurred at 12 weeks; 57th research day. They were weighed at 12 hours after slaughter to determine their cold carcass weight.

Statistical analyses

MS Excel and the R-Studio were used for the data analysis of live weight gain and cold carcass weights, given as the mean ± standard error (SE). Significance was tested by applying the *Student t-test*. Values of less than 0.05 ($p < 0.05$) were considered significant.

RESULTS AND DISCUSSION

During the initial health exam at 4 weeks of age the heart rate, respiratory rate, body temperature were recorded. No significant differences were identified among the control and synbiotic groups and all health indicators were within the normal range.

Throughout the research, the feces were inspected and the consistency recorded in points for each animal. Watery fecal consistency or diarrhea were never observed in any of the calves during the complete study. In all animal groups, softer than normal feces were observed during the 6th week of life. This was most likely due to the addition of fodder to the daily ration resulting in or promoting adaptive changes of digestive juices and microorganisms to the new feed product.

As shown in Fig. 1, after 8 weeks of age, when the calves had become used to the new feed product, calf fecal consistency stabilized in all groups, in a few individual animals softer than normal feces consistency, species-specific, was observed.

The highest fecal consistency number was recorded from the 5th –7th week when feces were softer than normal. This was most likely due to the introduction of synbiotics at the initial stage of the research that coincided with the 6-week life period of the calves.

Throughout the study, the greatest number of calves which had softer than normally soft feces were observed in the control group. In all three synbiotics groups, starting with the 7th week of life, the number of cases with softer than normal feces gradually decreased. The animals, which received 12 g of synbiotics (6 g of inulin), had the most stable fecal consistency among all groups through the study. Control group animals had the most liquid feces with the highest point number in comparison with the other groups.

In the synbiotic group with the lowest intake of inulin, fecal consistency reached 0.55 points (within normal limits) during the 9th week of life, but in the other two synbiotic groups this occurred later, by the 10th week. It may have been easier for calves to adapt to lower doses of inulin.

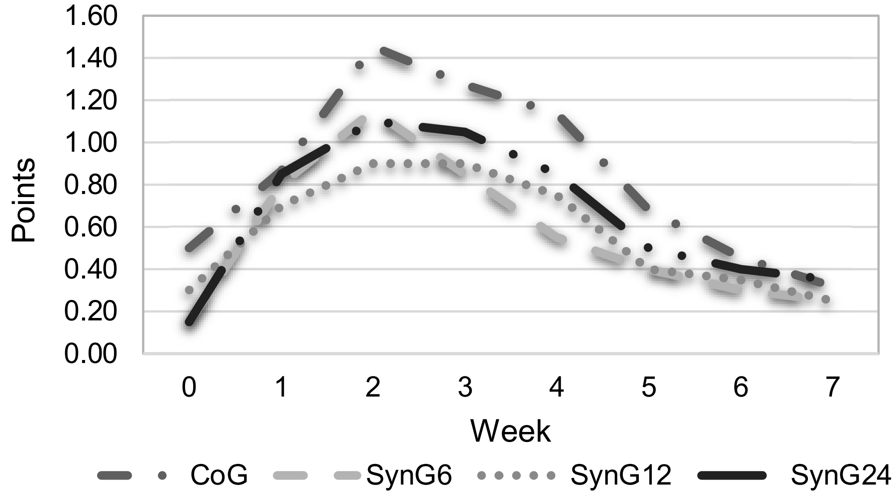

Figure 1. Comparison of the fecal consistency between calf groups from 5 to 12 weeks old (Calculating Values in 0–3 Points by Larson, 1977).

Table 2 displays per group: the average weight at 3 intervals, the total average weight gained during the study, and the average cold carcass weight on the 57th day. The average daily weight gain was also different among all synbiotic groups and the control group ($p < 0.01$) in which the control group had the lowest weight 82.18 kg of all groups.

Table 2. Calf growth dynamics by weight and cold carcass weight

Group	Average weight of calves (kg) at 3 research intervals			Average daily weight gain, kg	Cold carcass weight, kg
	first day	28th day	56th day		
CoG	50.80 ± 1.25	69.09 ± 5.97	82.18 ± 6.53	0.581 ± 0.13	42.6 ± 6.88
SynG6	51.00 ± 1.25	75.40 ± 4.35*	100.10 ± 3.14**	0.876 ± 0.06**	49.7 ± 2.41**
SynG12	51.70 ± 1.0	77.40 ± 1.65**	103.00 ± 2.26**	0.916 ± 0.09**	51.9 ± 1.64**
SynG24	52.13 ± 1.73	74.63 ± 1.19	99.96 ± 3.36**	0.854 ± 0.09**	49.6 ± 1.85**

*$p < 0.05$ compare to CoG; **$p < 0.01$ compare to CoG.

The SynG12 group had the greatest weight gain at 103.00 kg, ($p < 0.05$). This proves that the best inulin effect is achieved with the medium amount (or with 12 grams Jerusalem artichoke powder). This result may be due to firmer fecal masses observed throughout the study when compared to the other groups and due to the optimal combination of probiotics and prebiotics, which provides full digestibility of feed and improved intestinal function. Excessively high doses of prebiotics have been shown to decrease digestive function and the digestibility of feed. Weight gain is insignificantly but consistently higher in group SynG6 (100.10 kg) than in SynG24 (99.96 kg).

Most researchers studying synbiotics report that these supplements improve feed digestibility increasing the bioavailability of nutrients for growth and development. (Hasunuma et al., 2011, Zábranský et al., 2015; Marcondes et al., 2016; Dar et al., 2017; Geigerová et al., 2017).

Average weight gain per day was 0.630 kg in the control group. Daily weight gain higher was in the SynG12 group at 0.916 kg ($p < 0.01$). Daily weight gain was greater in the SynG12 group ($p < 0.05$) than in SynG24 (0.854 kg) and SynG6 (0.876 kg). The average daily weight gain in SynG24 and SynG6 was not significantly different;

however, slightly greater in SynG6. Research by Jatkauskas & Vrotniakiene (2010), showed that calves fed probiotics during the transition period to ruminant status had a 9.4% higher weight gain, than the control animal group which did not receive probiotics.

Throughout the study period, the smallest weight increase was observed in control animals, the greatest - in SynG12 (Fig. 2). The greatest increase in weight was observed during the middle of the study period in 6–8 week old calves when weight increased by an average of 14 kg in two weeks in each synbiotic group. During this period the weight gain of CoG was only 8 kg which was also the average weight gain for CoG throughout the study. The lowest weight increase among the synbiotic groups was observed in the first two study weeks. This is most likely due to the addition of the new synbiotic supplements.

Simon et al. (2001) in their study found that when feeding a probiotic, *Enterococcus faecium*, a significant weight gain was not seen in comparison with the control animals. This study proved that adding inulin with the *Enterococcus faecium* bacteria to the feed, significantly greater weight gain occurred in all the synbiotic groups as compared to the control group (Fig. 2).

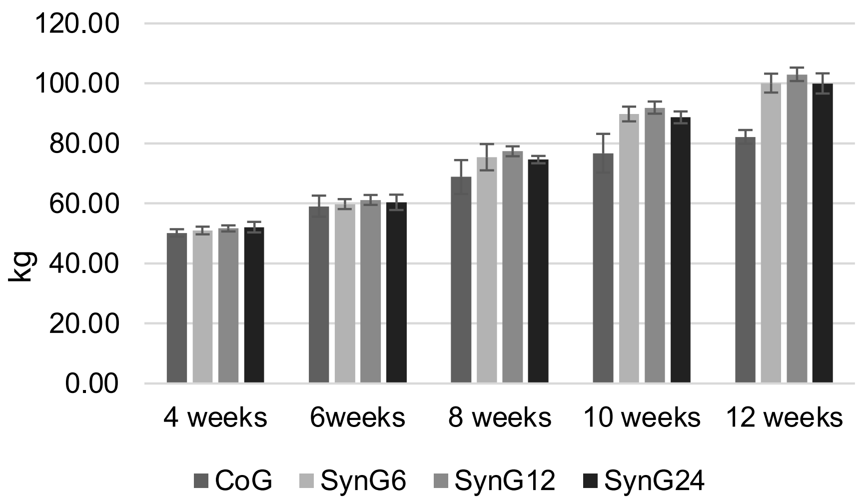

Figure 2. Average weight of calves on different study weeks in different groups.

Roodposhti & Dabiri (2012) found that calves fed with different synbiotics, had the highest food consumption and weight gain when compared to the control group (Marcondes et al., 2016).

Chaucheyras-Durand & Durand (2010), suggest that probiotics as a part of a synbiotic mix, begin colonization of the colon and increase the growth of beneficial bacteria, such as *Enterococcus faecium, Lactobacillus* and *acidophilus* which reduce the total number of pathogenic bacteria, thus securing for themselves a stable place in the colon. Prebiotics, under the influence of these beneficial bacteria, get fermented, increasing the total number of beneficial bacteria while reducing the pathogenic bacteria. Moreover, the fermentation process produces volatile fatty acids that improve animal energy usability and change the intestinal morphology (Roodposhti & Dabiri, 2012). In this study feeding beneficial bacteria, *Enterococcus faecium*, together with prebiotics, most likely increased the total number of bacteria. Inulin provides nutrients for microorganisms in the colon. Perhaps, feeding too much inulin (SynG24) changed the intestinal activity which may have resulted in less effective fermentation processes and feedstuff absorption. Therefore, the effect on weight gain was less than in the medium inulin dose (Fig. 3).

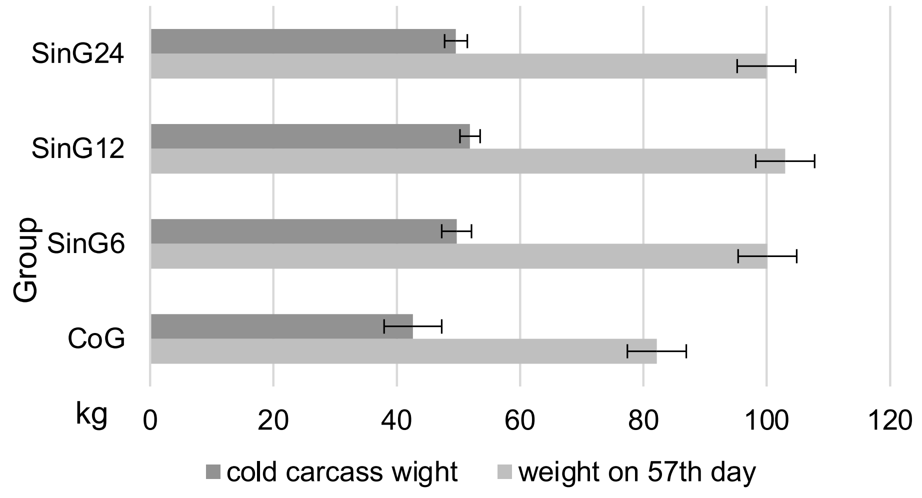

Figure 3. Comparison of mean values of calf live weight and cold carcass weight among control group and synbiotic groups on the 57th study day.

After the planned animal slaughter on the 57th day, the carcasses were chilled and weighed SynG12 carcass weight (52.3 kg) was higher ($p < 0.05$) than SynG6 (49.7 kg) and SynG24 (49.6 kg), although actual weight was on average, only 3 kg more. SynG12 carcass weight outcome was greater ($p < 0.01$) than the CoG, which was 9.3 kg.

The cold carcass weight of the CoG of animals was ($p < 0.01$) lower compared to the weight of other animal groups. Consequently, the average cold carcass weight of synbiotic groups is similar, SynG12 ($p < 0.05$) is higher than SynG6 and SynG24. Group SynG6 and SynG24 weights are similar.

CONCLUSIONS

Feeding synbiotics containing *Enterococcus faecium* and inulin to 1–3 - month old calves can stabilize their fecal consistency, increase average daily weight gain, total weight gain, and cold carcass weight outcome. Optimal inulin dose in this synbiotic mix was 12 g artichoke powder (6 g of inulin) per animal per day. In this case, the most gradual adaptation to the new feed supplement is observed that enables it to provide the most efficient species-specific fecal consistency and stable functioning of the digestive tract. It is possible to achieve the greatest increase in live weight gain and carcass weight outcome by feeding 12 g of Jerusalem artichoke powder together with the genus *Enterococcus* bacteria. Significantly higher weight increase per day (0.916 kg ± 0.09) has been gained by feeding synbiotics (24 g of Jerusalem artichoke powder and *Enterococcus faecium* 0.25 g).

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