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Comparative effect of different amount of inulin and symbiotic on growth performance and blood characteristics 12 weeks old calves

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Abstract. The study was focused on assessment of the effect of prebiotic inulin (from Jerusalem artichoke (JA) powder it contain ~50% of inulin) and mixed with probiotic preparation call synbiotic thereof on growth performance and blood parameters of milk calf. In total, 70 milk calves (50+/-5 kg; 23+/-5 d.old) were used in a 57-d experiment. The seven dietary treatments consisted of the control diet (CoG); diet CoG supplemented with different amount of prebiotics (PreG6; PreG12; PreG24; accordingly 6g, 12 or 24 g of JA) and three different amount of synbiotics (SynG6; SynG12; SynG24; accordingly inulin and probiotic 0.25 g Enterococcus faecium (2×10^9 CFU g^{-1}). Throughout the study prebiotic (PreG) and synbiotic (SynG) groups calf average daily gain (ADG) was increased ($p < 0.01$) than CoG, the end of study the highest ADG reached PreG12 than SynG12 and it was ($p < 0.01$) compare with CoG. At the end of te study PreG6 and SynG6;12 hematocrit was higher ($p < 0.05$), but PreG12;24 and SynG24 ($p < 0.01$) than CoG. Hemoglobin PreG and SynG12;24 was higher ($p < 0.01$) compare with CoG. WBC was lower ($p < 0.05$) PreG24, but there were no differences between synbiotic groups (SynG) compare CoG. PLT was ($p < 0.01$) higher PreG and SynG supplemented groups than CoG. Total protein PreG12; SynG6;12 ($p < 0.01$) higher compare CoG. Glucose PreG6;12 is lower ($p < 0.05$) than CoG. In conclusion, 12g and 24g of JA powder and the same amount ja powder mixed with probiotic Enterococcus faecium can improve the 4 to 12 weeks old calf performance and health status. However, new synbiotic didn't improve inulin action.

Key words: calf, inulin, *Enterococcus faecium,* growth, blood.

INTORDUCTION

Newborn ruminants are known to encounter potentially stressful situations during the first months of life, including morbidity, transportation, and weaning (Chashnidel et al., 2020). Stress can reduce immunity and increase the risk of disease (Salak-Johnson & McGlone, 2006), and for calves diarrhea is one of the most common causes of falls behind in growth and mortality (Gulliksen et al., 2009). To reduce this consequence there is search in different directions how to improve calf health. There was time when antibiotics were added to feed, but they stimulate the development of antibacterial resistance both in humans and in animals, and so the alternatives are searched (Jansons et al., 2011; Jonova et al., 2021). As some of the alternatives for several years are being investigated, prebiotics, probiotics and synbiotics, whose feeding to food-producing animals has increased in recent years (Hamasalim et al., 2016; Dar et al., 2017; Markowiak & Śliżewska, 2018; Tóth et al., 2020).

The prebiotic inulin is a polysaccharide belonging to the fructan group and is one of the most commonly used prebiotic in research. In the digestive tract, inulin is hydrolyzed and fermented to a substrate, which is used by the beneficial intestinal bacteria for their growth, so the numbers of those bacteria -such as Bifidobacteria and some species of bacteria Lactobacilli are increased (Han et al., 2014; Gupta et al., 2019; Jonova et al., 2021). Studies in ruminants have shown that inulin increases weight gain and induces a change in physiological blood measurements (Masanetz et al., 2010, 2011). There are studies showing that the use of prebiotics in ruminants substantially influence blood physiological parameters Chashnidel et al. (2020), reported that there is a significant ($p < 0.05$) difference in the amount of glucoses, BUN, total protein, albumin, globulin and IgG, also, Dar et al. (2017) observed substantially higher number of lymphocytes in the blood of calves which were fed prebiotics.

Combining prebiotics with probiotics call synbiotics that possible work better than prebiotics or probiotics alone (Shim, 2005; Hamasalim, 2016).

The aim of our study was to find out the impact on dairy calf daily gain as well as various blood hematological and biochemical parameters when using synbiotic got from the combination of inulin (obtained from Jerusalem artichoke) with probiotic (*Enterococcus faecium).*

MATERIALS AND METHODS

Study Herd, Housing and Feeding

Seventy randomly selected, 23 (± 5) days old clinically healthy male Holstein crossbreed calves, weighing 50 kg (± 5 kg), were randomly allocated to 7 groups. The study started when the animals had reached 4 weeks of age. The calves were kept in pen of 5, under the same +conditions and were fed twice a day, ~3.5 liter of whole milk per feeding. Calves were free-fed whole milk from a trough providing approximately 7L per calf daily. The pen for 5 calves was $25m^2$. 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. Bedding material was straw and pens were cleaned manually one time per day.

The calves were divided into 7 groups: control group (CoG, $n = 10$) was fed only whole milk, 3 with whole milk and different amount of prebiotic inulin (PreG6 = 6 g; PreG12 = 12g; PreG24 = 24 g of Jerusalem artichoke powder (JA) it contain ~50% of inulin) 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)). Inulin powder and probiotic were added to the first one of the two daily milk feedings.

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). There were no serious health problems observed during the experiment.

Blood sampling

The blood samples of calves were taken from *v.jugularis* externa on the 0, 28th and 56th day of the research before morning feeding. The level of total protein, albumin and glucose as well as blood morphology: red blood cells, white blood cells, platelets, hemoglobin and hematocrit were determined.

Blood samples for biochemical examination were taken from the calves into vacuumed sample tubes and samples were centrifuged at 3,500 rpm for 10 minutes to separate the serums. Resultant serums were then transferred to Eppendorf tubes and preserved at -23 °C until the time of analysis in the laboratory of Veterinary Hospital of Latvia University of Life Sciences and Technologies. Blood glucose measurements were performed on blood samples without preservatives by the *Freestyle Optium*. A drop of blood samples was placed on the test strip. Single-use reagent strips were used. The study lasted 8 weeks or 56 days.

Statistical analyses

Descriptive statistics for resultant data were expressed in means and standard errors. MS Excel and the R-Studio were used for the data analysis of live weight 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

Effects of prebiotic and synbiotic supplements on performance of Holstein calves are provided in Table 1. Calves receiving prebiotic inulin (PreG) in amount 3, 6 and 12 g day^{-1} per head and mixed with probiotic call synbiotic (SynG) showed clearly higher ($P < 0.01$) body weight in first 12 weeks of life than calves from control group (CoG) (Table 1). It was confirmed by higher daily weight gains of calves that whole milk were supplemented by prebiotic and synbiotic (Table 1).

Table 1. Average daily weight gain of calves in different research periods

Group	Average weight of calves (kg) at 3 research intervals			Average daily weight gain (kg)		
	0.–28. day	28.–56.day	0.–56. day	0.–28. day	28.–56. day	0.–56. day
CoG	18.2 ± 5.06	12.4 ± 5.09	31.2 ± 8.44	0.67 ± 0.20	0.44 ± 0.18	0.56 ± 0.15
PreG6	26.5 ± 1.90**	18.2 ± 3.58**	44.7 ± 4.03**	0.95 ± 0.07**	0.65 ± 0.13**	0.80 ± 0.07**
PreG12	29.0 ± 1.88**	23.3 ± 5.09**	52.3 ± 3.68**	1.03 ± 0.06**	0.83 ± 0.17**	0.93 ± 0.07**
PreG24	25.2 ± 7.36*	22.9 ± 5.09**	48.1 ± 4.18**	0.90 ± 0.26**	0.82 ± 0.18**	0.86 ± 0.08**
SynG6	24.4 ± 3.68**	24.7 ± 4.69**	49.1 ± 2.81**	0.86 ± 0.19**	0.88 ± 0.16**	0.88 ± 0.03**
SynG12	25.7 ± 1.57**	25.6 ± 2.32**	51.3 ± 2.71**	0.91 ± 0.12**	0.92 ± 0.08**	0.92 ± 0.02**
SynG24	22.5 ± 1.65	26.1 ± 3.70**	47.6 ± 4.28**	0.93 ± 0.13**	0.93 ± 0.13**	0.85 ± 0.03**

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

Started with 28th research day hematocrit (HCT) was higher ($p < 0.01$) all prebiotic groups animal compare to CoG, in research 56th day PreG6 group was higher ($p < 0.05$) HCT, but PreG12, PreG24 ($p < 0.01$) higher HCT compare to CoG. Control group hemoglobin (HGB) in 28th research day was lower ($p < 0.01$) than PreG24 and lower ($p < 0.05$) than PreG6 group. End of the study in day 56th all prebiotic groups HGB was higher ($p < 0.01$) compare to CoG. Highest erythrocytes (RBC) level was group PreG12 and in 28th and 56th research day was higher ($p < 0.01$) than CoG. PreG24 group RBC was higher ($p < 0.01$) in the 28th research day and ($p < 0.05$) end of the research (Table 2).

Table 2. Blood hematology variables of prebiotic groups calves on 0; 28th and 56th research days (mean ± *SD*)

	Research day	Group CoG	PreG6	PreG12	PreG24
HCT (%)	0	22.5 ± 4.26	24.1 ± 4.51	23.8 ± 3.51	24.1 ± 4.48
	28	18.4 ± 5.53	24.0 ± 4.02**	24.3 ± 2.34**	26.8 ± 1.56**
	56	18.6 ± 3.29	22.6 ± 4.62*	25.8 ± 2.30**	25.7 ± 1.01**
HGB (g/dl)	0	9.1 ± 1.21	9.0 ± 1.55	9.2 ± 1.30	9.2 ± 1.29
	28	8.2 ± 1.68	9.8 ± 1.41*	9.2 ± 0.81	10.2 ± 1.71**
	56	8.6 ± 0.75	9.8 ± 1.12**	9.7 ± 0.71**	10.5 ± 1.79**
RBC (x1012/L)	0	6.9 ± 1.51	7.6 ± 1.49	8.0 ± 0.70*	7.7 ± 0.94
	28	6.7 ± 1.20	8.5 ± 1.22*	7.9 ± 1.09**	8.2 ± 0.93**
	56	7.2 ± 0.91	7.6 ± 1.29	8.3 ± 0.45**	7.8 ± 1.26*
WBC (x109/L)	0	8.9 ± 1.07	9.1 ± 1.41	8.9 ± 1.13	8.8 ± 0.92
	28	9.5 ± 1.46	9.3 ± 1.44	9.7 ± 1.14	8.8 ± 1.80
	56	10.9 ± 1.29	11.7 ± 1.67	9.5 ± 1.77*	8.7 ± 1.15**
PLT(x109/L)	0	812.9 ± 216.66	774.6 ± 258.98	566.5 ± 251.79**	714.8 ± 187.80
	28	992.2 ± 88.04	592.9 ± 180.35**	657.8 ± 173.69**	660.5 ± 113.87**
	56	687.1 ± 211.78	591.7 ± 124.80**	649.0 ± 113.68**	601.8 ± 110.61**

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

Leucocytes (WBC) significantly difference was in 56th research day were PreG12, Pre24 higher ($p < 0.05$) compare to CoG.

Platelets was influenced by dietary treatments. Control group platelets (PLT) was significantly higher ($p < 0.01$) starting from 28th study day (Table 2, 3).

Table 3. Blood hematology variables of synbiotic groups calves on 0; 28 and 56 research days (mean ± *SD*)

	Research day	Group CoG	SynG6	SynG12	SynG24
HCT (%)	0	22.5 ± 4.26	22.5 ± 2.96	23.9 ± 3.63	25.9 ± 3.46*
	28	18.4 ± 5.53	22.5 ± 3.87*	23.2 ± 5.98*	25.1 ± 4.72**
	56	18.6 ± 3.29	21.8 ± 3.60*	22.7 ± 5.31*	24.9 ± 4.39**
HGB(g dl^{-1})	0	9.1 ± 1.21	8.8 ± 1.01	9.1 ± 1.24	10.2 ± 1.04*
	28	8.2 ± 1.68	9.3 ± 0.70*	10.1 ± 0.92**	10.2 ± 0.74**
	56	8.6 ± 0.75	8.4 ± 0.85	9.1 ± 1.11**	9.9 ± 0.86**
RBC(x1012/L)	0	6.9 ± 1.51	7.4 ± 1.44	7.6 ± 1.81	8.0 ± 1.77
	28	6.7 ± 1.20	7.4 ± 0.83*	7.9 ± 1.21**	9.1 ± 0.82**
	56	7.2 ± 0.91	7.5 ± 1.23	7.4 ± 1.60	7.7 ± 1.56
WBC (x109/L)	0	8.9 ± 1.07	9.2 ± 1.56	9.9 ± 1.57*	9.9 ± 1.69
	28	9.5 ± 1.46	11.9 ± 1.81*	10.9 ± 2.52	9.8 ± 1.51
	56	10.9 ± 1.29	8.6 ± 1.73**	9.7 ± 2.50	10.2 ± 2.70
PLT(x109/L)	0	812.9 ± 216.66	870.1 ± 221.61	928.5 ± 168.51	668.3 ± 212.35
	28	992.2 ± 88.04	921.0 ± 202.85	716.7 ± 210.14**	673.4 ± 185.26**
	56	687.1 ± 211.78	703.0 ± 195.95*	666.2 ± 203.74**	649.0 ± 218.28**

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

All synbiotic groups HCT starting from 28th research day was higher ($p < 0.05$) compare with CoG, SynG24 it was significantly ($p < 0.01$) higher, but SynG6;12 ($p < 0.05$) higher than CoG (Table 3). In the end of the research. SynG6;12 it was ($p < 0.05$) higher, but SynG24 ($p < 0.01$) higher than CoG. HGB SynG12;24 starting from 28th research day was significantly higher ($p < 0.01$). In the middle of the research RBC all synbiotic groups was higher ($p < 0.05$) compare to CoG, but in 56th RBC was not affected by treatment (Table 3).

Leucocytes SynG6 group has higher ($p < 0.05$) than CoG in day 56th it was lower ($p < 0.01$) compare to CoG.

PLT was significantly ($p < 0.01$) influenced by inclusion of middle and high amount of synbiotic (SynG12, SynG24) (Table 3). Inclusion of middle (6 g) and high (12 g) amount of inulin and it combination with probiotic resulted ($p < 0.01$) in a significant increase in total protein (TP) concentration (Table 4).

Table 4. Blood clinicochemical variables of calves on 0, 28th and 56th research days (mean ± *SD*)

	Research day	Group						
		KoG	PreG6	PreG12	PreG24	SynG6	SynG12	SynG24
TP	0	60 ± 1.5	59± 2.9	56 ± 3.32**	56 ± 3.8**	58 ± 2.7	60 ± 2.3	59 ± 2.3
(g L^{-1})	28	54 ± 3.8	54 ± 4.6	51 ± 10.9	58 ± 2.9**	54 ± 3.9	55 ± 2.7	54 ± 4.0
	56	58 ± 2.2	57 ± 2.4	63 ± 1.5**	61 ± 2.2**	58 ± 2.9	62 ± 2.5**	63 ± 3.2**
Alb	0	41 ± 1.1	42 ± 1.5	39 ± 1.9*	39 ± 1.2*	40 ± 2.5	42 ± 0.6**	41 ± 1.2
(g L^{-1})	28	37 ± 2.6	37 ± 2.8	35 ± 1.0**	36 ± 1.7	35 ± 0.7*	39 ± 1.5	37 ± 2.3
	56	39 ± 2.1	38 ± 1.1	37 ± 1.2*	38 ± 1.9	38 ± 1.5	38.1 ± 1.7	39 ± 1.9
Gl	0	5.5 ± 0.6	5.3 ± 0.6	5.0 ± 0.5	5.9 ± 1.5	5.7 ± 0.4	5.8 ± 0.3	5.3 ± 0.5
(mmol L^{-1})	28	5.9 ± 0.7	5.8 ± 0.6	4.5 ± 0.4**	4.6 ± 0.8**	5.8 ± 0.4	4.9 ± 0.4	4.6 ± 0.4
	56	4.9 ± 0.7	4.7 ± 0.8	4.1 ± 0.4**	4.5 ± 0.9*	4.8 ± 0.6	4.6 ± 0.5	4.2 ± 0.3**

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

As given in Table 4, serum Albumin (ALB) concentration was not influenced by prebiotic and synbiotic dietary treatments. Additionally, substitution of 6 g inulin markedly ($p < 0.01$) decreased the glucose level in the 28th and 56th day of research. High dose of inulin (12 g) decrease ($p < 0.05$) glucose level in the 28th and 56th day of the research. As given in Table 4, glucose concentration was not influenced by synbiotic dietary treatments, omitting high dose synbiotic group (SynG24) it was significantly ($p < 0.01$) decrease compare to CoG in the end of the study.

DISCUSSION

This study proved that adding inulin and inulin combination with the *Enterococcus faecium* bacteria to the feed, significantly greater weight gain occurred in all the prebiotic and synbiotic groups as compared to the control group. The present results are similar with author research (Adams et al., 2008; Masanetz et al., 2010; Król, 2011; Markowiak & Śliżewska, 2018; Jonova et al., 2021).

In our and other researchers' studies, the age of calves is variable, so, as has written Klinkon & Ježek (2007), the hematological and biochemical calves. Blood parameters are especially affected at the time when dry food (hay, concentrate) is fed after milk feeding. The main function of erythrocytes is gas transportation, supplying cells with oxygen and ensuring that they have sufficient hemoglobin levels (Awodi et al., 2005). In our study, number of erythrocytes in calves. PreG12;24 increased, which could be explained in a way that inulin effects the attraction of iron ions to cells (Tako et al., 2008). Throughout the study, control group animals generally had lower levels of hematocrit, erythrocytes, and hemoglobin than animals in the prebiotic groups. The lowest hemoglobin levels in control group were observed on day 28th of study, which coincides with studies by other authors (Mohri et al., 2007; Dar et al., 2017). Also in their study the lowest hemoglobin levels in calves were observed at the same age, which then tended to increase again. Such trend was not observed in calves of the prebiotic groups, which could indicate the better iron absorption. In CoG animals the HCT at 28th and 56th day is below normal, indicating lower oxygen saturation to cells in this group of animals. Masentez et al. (2011) in his study had not found significant differences in the total number of red blood cells between the prebiotic group and control group animals, but there was significantly higher level of hemoglobin in the groups were inulin was fed in comparison with control groups were lactulose was used. This coincides with the results of our study, where the medium and high doses of inulin feeding increases the level of HGB level.

Also, Król (2011) study with calves which received inulin 3 g day^{-1}, on day 56th the level of hemoglobin in the blood was significantly ($p \leq 0.05$) higher than the other groups, which are similar to the results of our study and approve the inulin intake positive effect on HGB raising in calves.

In our study, leukocyte counts showed the most stable results in the PreG24 group, CoG and PreG6 animals, with the most in the final stage of the study on the 56th day. At the end of the study, animals of the PreG24 group had less than the control group, but there were no significant differences observed during the rest of the study, what is similar to (Roodposhti & Dabiri, 2012). Also, Król (2011) in his study where was fed inulin 6 g day^{-1} on the 56th day of the study obtained a higher ($p \leq 0.05$) amount of leukocytes than in the other groups, indicating that feeding higher doses of prebiotics increases the number of leukocytes in the body. This may be due to an inflammatory response in the intestines to a high dose of prebiotics. Also El-Mehanna1et al. (2017) in their study found that the total white blood cell count is higher in the prebiotic and synbiotic group than in the control group, which would confirm this theory.

The thrombocytes count in control group animals were significantly higher throughout the study compared to the prebiotic and synbiotic group. Lower platelet counts reduce the risk of blood clots and cardiovascular disease, which is due to the intake of high-fiber foods such as prebiotics (Bagger et al., 1996). Król (2011) found that the control group animal's platelet count is three times higher than for calves which were fed prebiotics.

During postnatal development, the age of calves is cited as a major factor influencing total protein levels, emphasizing that it is lower in calves than in cows (Shil et al., 2012). Tao et al. (2015) fed beta glucans to calves and concluded that they did not affect total protein level, as well as albumin. In contrast, our study showed that calves that received a middle dose of inulin (12 g day^{-1}) has a higher ($p < 0.01$) total protein level than CoG, at the end of the study PreG12, SinG6 and SinG12 groups ($p < 0.01$) was higher than CoG. Albumin, in our study, reaches the lowest point on the day 28th which coincides with the time of fecal mass dilution, which is one of the possible causes of the decrease of albumin.

It is observed that glucoses in blood serum in calves has higher than in cows, which could be explained by the fact that in calves the stomach initially functions similarly to monogastric animals. When calves become older, the level of glucoses is decreasing (Shil et al., 2012), which is similar in our study. Feeding different doses of prebiotics or probiotics, several authors mention that no significant differences in serum glucose levels were observed compared to control group and experimental groups (Adams et al., 2008; Dar & Para, 2019). However, in our study, the animals of PreG12 group on the days 28th and 56th had ($p < 0.01$) lower glucose levels than control group animals. Similarly, in SinG24 it is significantly ($p < 0.05$) lower than in control group. El-Mehanna1 et al. (2017) in studies with lambs fed by FOS and synbiotics, hadn't observed, significant differences ($p > 0.05$) in glucose level. Similarly, Ghorbani et al. (2002) combining the use of *Propionibacterium* and *Enterococcus faecium* did not have any significant differences with in comparison with the control group.

CONCLUSIONS

By feeding inulin 6 and 12 g day^{-1}, it is possible to achieve a higher weight gain, which is significantly higher than in control group. Medium and high doses of inulin affect the levels of red blood cells, hemoglobin and hematocrit in the blood of animals. Inulin feeding can affect blood parameters of calves of transitional feeding age so also do the synbiotics. However, the results with feeding synbiotics were not providing conclusive results on the improvement of animal's blood parameters.

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