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EFFECTS OF USING PROBIOTICS ON CALVES GROWTH RATES AND HEMATOLOGIC PROFILE

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Abstract

The aim of the current study was to evaluate the effects of using Enterococcus faecium as probiotic on dairy calves' growth rates and health status. During a 42 days trial, sixteen un-weaned Romanian Black Spotted calves were homogenously assigned in two groups: C (n = 8, control) and E (n = 8, treated with 2 x 10^8 CFU/ml of Enterococcus faecium). Body weight, blood sampling and diarrheic score were assessed at 0, 14, 28 and 42 days following administration of the probiotic. At the end of the trial, probiotic administration affected positively the experimental group, having a higher average daily gain with 23.96% compared to the control (ADG = 763.35g vs. 616.28g). Probiotic supplementation had no significant effects (P>0.05) on the hematologic profile and on calves health status. However, the prevalence of diarrhoea was higher in group C (32%) compared with group E (16%). Results suggest that throughout the use of Enterococcus faecium in un-weaned calves diets, the growth rates are improved and this could represent an approach in the control and prevention of enteric diseases.

***Key words**: antibiotics, calves, health, probiotic, weaning.*

INTRODUCTION

In order to support dairy calves growth and development, nutrition and the health status are regarded as the main influencing factors (Malmuthuge et al., 2015). At the same time, dairy farmers express interest in accelerating the growth of calves (Liu et al., 2019), improving feed efficiency conversion (Turiello et al., 2020), while reducing the overall production costs (Xiao et al., 2020). Despite progresses reached in intensive rearing and feeding technologies, dairy calves' morbidity and mortality are still causing important economic losses (Raboisson et al., 2016). Moreover, un-weaned calf morbidity and mortality are frequently associated with enteric diarrhoea (Hulbert et al., 2016).
It was shown that induced stress factors such as isolation, ear-tagging, vaccination and weaning practices, have a significant influence on health and growth rates of calves, and the overall productivity (Mikus et al., 2020).
Nutrition and feeding strategies were shown to influence both growth and health of un-weaned dairy calves (Meale et al., 2016). In calves nutrition, antibiotics have been an important measure to balance health status and feed efficiency in intensive dairy production systems. Although both antibiotic feed additives and prevention antibiotics were banned in the EU starting year 2006, antibiotic traces were found in calves milk diets (Pogurschi et al., 2015; Chiesa et al., 2016). In the same time, avoiding bio-resistant microorganism induction and sustaining natural immune system (adapted or innate) could be the main key in controlling early bacterial infections (Van den Honert, 2019; Renaud et al., 2019; Flores et al., 2019).
Probiotics used as feed supplements are supported by the EU as potential alternatives to antibiotics (Regulation No. 767/2009). Among microorganisms with potential probiotic effects, *Enterococcus faecium* spp. were acknowledged as components of gastric

microbiome (Holzapfel et al., 2017), abundantly found in soil, water and waste (Aziz et al., 2019). In the last years, *Enterococcus faecium* spp. have gain interest for their beneficial probiotic attributes expressed in fermented foods (Marcondes et al., 2016; Brcina et al., 2019; Schittler et al., 2019), with a few strains being reported as bacteriocin producers (Qiao et al., 2019) and having significant antipathogenic traits (Hanchi et al., 2018). *Enterococcus faecium* NCIMB 11181 is used in pharmaceutical and para-pharmaceutical formulations, given that this strain targets pathogenic microorganisms, improving nutrient availability and the overall health status (Cangiano et al., 2020; Kayasaki et al., 2021).

The aim of the current study was to evaluate the effects of *Enterococcus faecium* NCIMB 11181 probiotic on un-weaned dairy calves growth rates, haematological profile and diarrhoeic prevalence.

MATERIALS AND METHODS

Sixteen un-weaned Romanian Black Spotted purebred calves, were homogenously assigned in two half-siblings' groups, balanced for sex, age and body weight during a 42 days trial, as follows:

- experimental group (E) nil per os (NPO) probiotic administration (n = 8);
- control group (C) with no probiotic treatment (n = 8).

Probiotic administration was performed daily during the first 28th days of the trial, with *per os* administration of 2 x 10^8 CFU/ml of *Enterococcus faecium* NCIMB 11181 (commercial probiotic strain). The following 14th monitoring days were used to observe probiotic post administration effects on calves development and haematological profile.

Calves were housed in individual hutches on deep straw bedding. During the first 3 days of life, calves were fed with 4 kg of colostrum per day, in two equal meals at 12 hours intervals. After colostrum administration, calves received a diet consisting out of 6 kg of milk substitute, into two equal meals per day [Eurolac 22/16, Schills, (125 g/L)]. Starting 10 days of life, the calves were offered unrestricted access to water, starter concentrates and alfalfa hay until the age of 3 months, when weaning took place.

Live body weight of calf was evaluated using a weighing scale platform, on the 0, 14th, 28th and 42nd days of experimentation.

Calves blood samples were collected on the 0, 14th, 28th and 42th day of the experiment. Samples from all calves were collected from the jugular vein, using vacutainer tubes with K_3EDTA (Kima®, Italy) containing 3.6 mg EDTA/K3 per ml of blood collected. Haematological determinations were done using a haematology analyser (Diatron, Abacus Junior Vet, Hungary). The hematologic screened parameters were: red blood cells count (RBC), red cell distribution width (RDW), haemoglobin concentration (HGB), mean corpuscular haemoglobin (MCH), platelets count (PLT), platelets percentage (MPV), platelets distribution width (PDW), total white blood cells count (WBC), lymphocytes count (LYM), monocytes count (MON), neutrophils count (NEU), haematocrits (HCT) and mean corpuscular volume (MCV).

During the 42 days of trial, diarrhoeic prevalence (PREV%) was registered daily. Diarrhoeic cases were reported and diagnosticated by the experimental farms' veterinarian.

Ethics statement

The research activities were performed in accordance with the European Union's Directive for animal experimentation (Directive 2010/63/EU). Use of animals and the procedures performed in this study were approved by the Scientific and Ethics Committee of the Research and Development Institute for Bovine Balotesti.

Statistical analysis

Statistical evaluation of live body weight and average daily gain were expressed as descriptive statistic, mean ± standard error of the mean. Although, calculation such as the prevalence rates were performed for the enteric cases. Hematological parameters were calculated using the analysis of variance (one-way ANOVA), at 0.05 level of significance.

Table 1. Chemical and amino-acid composition of calf starter concentrate and milk replacer

Chemical parameters	Units	Calf starter	Milk replacer
Nutritive units	UNL/kg	0.99	n.a.
Crude protein	%	18.5	22
Crude fat	%	1	16
Crude fibre	%	9	0.9
Methionine	%	0.36	n.a.
Lysine	%	0.9	n.a.
Calcium	%	2.69	n.a.
Phosphorus	%	0.69	n.a.
Salts	%	0.9	n.a.

RESULTS AND DISCUSSIONS

Calves live body weights are presented in Figure 1. At the beginning of the experimental trial, average live body weight ± SEM of calves were similar (C = 66.00 ± 7.27 kg and E = 66.96 ± 9.71 kg). Supplementing the calves diet with *E. faecium* had positive results, resulting in an overall average body weight higher with 7.9% in the experimental group (99.81 ± 9.74 kg), compared with the control group (92.50 ± 12.74). The increase in live body weight could be associated with antipathogenic specific attributes, enhancing the benefic bacteria populations and competitional pathogenic exclusion (Grigore et al., 2020). Our results are comparable with those of Sahu et al. (2019), which found a significant increase among calves body weights when using a solid *E. faecium* probiotic (70 mg/kg feed). High body weight gain shows that probiotic supplementation has positive effects, mainly based on enhancement of nutrient biodisponibility and benefic bacteria proliferation in the gastrointestinal microflora (Malmuthuge, 2017). Probiotic administration has proven positive effects after the first 14 days of administration, indicated by the body weight gain differences (2.81%) among control (73.43 ± 8.04 kg) and the experimental group (75.50 ± 10.19 kg). At 28 days, body weights were higher (3.37%) in the experimental group (86.94 ± 10.84 kg), compared with the control (84.10 ± 9.08 kg), indicating that long time-low concertation probiotic administration could support and enhance calves growth, in accordance with results published by Radzikowski (2017). In addition, important factors such as individuality, husbandry conditions and nutrition management are directly affecting calves performance (Irimia et al., 2020) and it is recommended to implement measures in order to support and promote calves health and development.

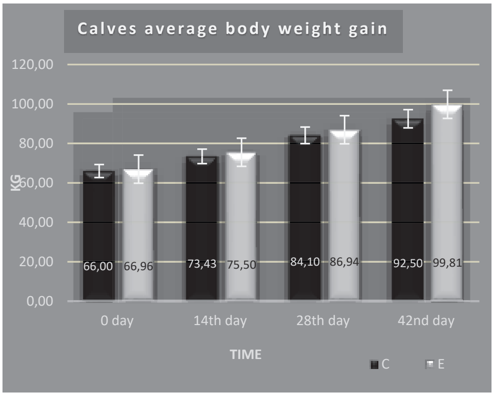

Figure 1. Calves average live body weight on days 0, 14, 28 and 42 of the experimental trial

Average daily gains are presented in Figure 2. At the end of the 42 days trial, probiotic administration affected positively the E group, having a higher average daily gain with 23.96% compared to the control (ADG = 763.35 g vs. 616.28 g). Our results show that probiotic administration in calves diets have positive effects (656.73 ± 78.78 g) starting the first 14th days of administration, leading to higher values with 14.98 g/head/day, compared with the control group (571.15 ± 62.76 g). In addition, Kelsey and Colpoys (2018) had similar findings when administrating a multi-strain probiotic containing *E. faecium* in calves diet, during the first 3 weeks of experimentation. On the contrary, Salazar et al., 2019 studied the *E. faecium* probiotic supplementation (70 mg/kg solid feed) and found no significant effects between control and the experimental group on average daily gain during the preweaning period. In addition, the same authors found that after weaning, calves treated with probiotic had lower body weights compared to their weaning weights, underlaying that probiotics had no residual effects on calves growth performance. Furthermore, Marcones et al. (2016) indicate no significant difference between pre-weaned Holstein calves growth performance fed with mixed prebiotics-probiotics and the control group.

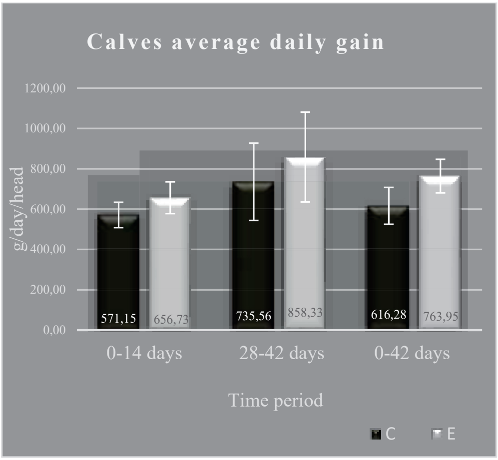

Figure 2. Calves average daily gain 0-14, 28-42 and 0-42 intervals of the experimental trial

Calves hematologic profile are displayed in Table 3. All haematological resulted data ranged within the reference values limits provided by Merck Veterinary Manual (2014) and Schalm's Veterinary Haematology (2011) (Table 2.). *E. faecium* dietary supplementation had no significant difference (P>0.05) between the control and experimental groups, indicating that probiotics might not interfere with the haemologic profile and could not modulate within leukocyte counts and leukocyte haematologic formula. RBC, HGB, HCT RDW, MCV and MCH were not influenced by dietary *E. faecium* supplementation (P>0.05), thus haematopoiesis related as red blood cells, haemoglobin and haematocrit could not be affected or stimulated by the probiotic treatment. Moreover, pre-weaned calves demonstrated a decreased activity of RBC before parasitic infestation (Emery et al., 2020) or stress challenges (Dahl et al., 2020). Low levels of RGB and HGB could be a natural response to anaemia, for example Fe^{+} or vitamin A deficiencies. However, calves raised on milk or milk replacer diets tend to present lower levels of RBC, as outlined by Weiss and Wardrop (2011). Platelets counts (PLT) and platelets haematologic formula (MPV, PDWc) were not influenced by *E. faecium* probiotic strain (P>0.05), indicating a physiologic and homeostatic health status without lesions, blood loss or abnormal cell encounter, in accordance with results by Vorobyeva and Medvedev (2020).

Table 2. Calves haematologic reference values intervals according to Merck Veterinary Manual and Schalm's Veterinary Haematology*

Haematologic parameter	Merck Veterinary Manual	Schalm's Veterinary Haematology
WBC	4-12x10^9/L	5.1-13.3 x10^3/µL
LYM	45-75%	1.8-8.1 x10^3/µL
MON	2-7%	0.1-0.7 x10^3/µL
NEU	15-45%	-
RBC	5-10 x 10^{12} g/L	4.9-7.5 x 10^6/µL
HGB	80-150 g/L	8.4-12 g/dL
HCT	24-46%	21-30%
MCV	40-60 fL	36-50%
MCH	-	14-19%
RDW	-	16-20%
PLT	-	160-650 x 10^3/L
MPV	-	4.6-7.4fL

*Merck Veterinary Manual 10th ed. and Schalm's Veterinary Haematology reference intervals. Table revised and inspirated after Weiss and Wardrop (2011) and Bedenicki et al. (2014)

Calves diarrhoea remains the main reason for calves morbidity and mortality (Urie et al., 2018). During our research trial, none of the calves died. At the beginning of the trial (first 14th days) the prevalence rate in the experimental group was similar to the control. The second period (the following 15th days) the prevalence rate was higher in the control group, compared with experimental group. The third interval (29-42 days) had a similar pattern, with none of the *E. faecium* supplemented calves developing the disease. The overall calves diarrhoeic prevalence was higher in the C group (32%), compared with the E group (16%). Results suggest that throughout the use of *E. faecium* in un-weaned calves diets, the diarrhoeic episodes are reduced, alongside with the severity of the symptoms, which could help in order to maintain the anti-bio resistance and to use the antibiotics only on special needs and severities. Besides growth promoting rates, *E. faecium* improved the immune response and this could represent an approach in the control and prevention of enteric diseases.

Table 3. Evaluation of probiotic effect on Romanian Black Spotted dairy calves haematologic profile between 0 and 42nd days of administration

Item		$\overline{x} \pm SEM$		p-value
		Control	Experimental	
WBC (x10⁹/L)	*0*	9.52±0.82	10.14±1.35	0.73 (*N.S*)
	14th	9.58±0.64	9.31±0.72	0.78 (*N.S*)
	28th	8.47±0.70	10.39±0.47	0.07 (*N.S*)
	42nd	9.73±0.85	9.93±0.47	0.83 (*N.S*)
LYM (%)	*0*	5.38±0.80	5.05±0.58	0.74 (*N.S*)
	14th	5.64±0.54	5.45±0.38	0.78 (*N.S*)
	28th	5.58±0.40	5.97±0.62	0.58 (*N.S*)
	42nd	5.47±0.48	6.15±0.17	0.31 (*N.S*)
MON (%)	*0*	0.178±0.028	0.187±0.055	0.90 (*N.S*)
	14th	0.171±0.126	0.155±0.085	0.83 (*N.S*)
	28th	0.135±0.032	0.141±0.029	0.89 (*N.S*)
	42nd	0.191±0.155	0.183±0.069	0.93 (*N.S*)
NEU (%)	*0*	5.88±1.28	5.17±1.20	0.69 (*N.S*)
	14th	3.71±0.19	3.70±0.66	0.99 (*N.S*)
	28th	3.06±0.37	4.60±0.76	0.13 (*N.S*)
	42nd	4.87±0.94	5.12±0.90	0.85 (*N.S*)
RBC (10¹²/L)	*0*	8.06±0.37	8.80±0.33	0.15 (*N.S*)
	14th	8.48±0.30	8.88±0.30	0.35 (*N.S*)
	28th	8.85±0.29	8.95±0.33	0.82 (*N.S*)
	42nd	8.65±0.40	8.11±0.48	0.39 (*N.S*)
HGB (g/L)	*0*	9.27±0.33	9.5±0.45	0.69 (*N.S*)
	14th	9.35±0.33	9.21±0.33	0.77 (*N.S*)
	28th	9.43±0.26	9.15±0.22	0.44 (*N.S*)
	42nd	9.40±0.26	8.43±0.43	0.07 (*N.S*)
HCT (%)	*0*	26.28±0.91	28.19±1.51	0.29 (*N.S*)
	14th	27.31±1.10	27.84±1	0.74 (*N.S*)
	28th	27.89±0.92	27.08±0.74	0.51 (*N.S*)
	42nd	26.98±1.03	24.47±0.89	0.09 (*N.S*)
MCV (fL)	*0*	32.75±0.92	32±1.38	0.66 (*N.S*)
	14th	32.5±0.65	31.25±0.65	0.20 (*N.S*)
	28th	31.5±0.53	30.37±0.71	0.22 (*N.S*)
	42nd	31.5±0.94	29.5±1.38	0.25 (*N.S*)
MCH (%)	*0*	11.63±0.53	10.8±0.38	0.22 (*N.S*)
	14th	11.04±0.21	10.38±0.27	0.07 (*N.S*)
	28th	10.7±0.23	10.13±0.28	0.14 (*N.S*)
	42nd	11.1±0.74	10.55±0.65	0.58 (*N.S*)
RDW(%)	*0*	23.73±0.56	25.26±0.90	0.17 (*N.S*)
	14th	24.58±0.53	25.08±0.76	0.59 (*N.S*)
	28th	24.14±0.40	25.28±0.88	0.22 (*N.S*)
	42nd	26.21±1.01	26.23±0.90	0.99 (*N.S*)
PLT (x10⁹/L)	*0*	415.63±37.56	398.86±43.51	0.77 (*N.S*)
	14th	439.38±31.93	460.88±39.46	0.68 (*N.S*)
	28th	425.63±29.65	404.88±43.47	0.70 (*N.S*)
	42nd	442±33.40	464.5±44.36	0.69 (*N.S*)
MPV(fL)	*0*	5.69±0.17	5.49±1.70	0.42 (*N.S*)
	14th	5.56±0.09	5.46±0.12	0.50 (*N.S*)
	28th	5.43±0.04	5.38±0.06	0.42 (*N.S*)
	42nd	5.76±0.14	5.45±0.13	0.13 (*N.S*)
PDWc	*0*	32.96±0.43	30.52±1.36	0.11 (*N.S*)
	14th	32.99±0.65	31.49±0.77	0.16 (*N.S*)
	28th	31.98±0.31	30.71±0.72	0.13 (*N.S*)
	42nd	31.86±1.55	31.18±0.82	0.70 (*N.S*)

Current results are in accordance with those of Renauld et al. (2019), which showed that by using multi-strain probiotics (including the *E. faecium* strain), the duration and the resolution of diarrhoeic episodes was reduced. Contrary, Smidkova and Cziek (2018) research suggests that *E. faecium* treatment of new born calves (+12h *post-partum* until 14^{th} day of life) do not reduce the *E. coli* faecal counts.

CONCLUSIONS

The current study shows that supplementation of pre-weaned calves diets with *Enterococcus faecium* NCIMB 11181 probiotic strain could improve live body weight and average daily gain. At the same time, probiotic intervention might represent a viable control measure in order to mitigate enteritis symptoms and support calves health status recovery. More research is needed in order to elucidated the long-term effect of *E. faecium* on the calves growth rates, intestinal microbiota and health status.

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