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EFFECTS OF MULTI-STRAIN PROBIOTICS ADMINISTRATION ON GROWTH PERFORMANCE AND HEALTH STATUS IN DAIRY AND BEEF-DAIRY CROSSBREED CALVES

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Abstract

The aim of the current study was to evaluate the effects of multi-strain probiotic administration on dairy and crossbreed calves' growth performances and health status. The calves were homogeneously assigned into four groups, as follows: dairy experimental (E_d, n=12), dairy control (C_d, n=12), crossbreed experimental (E_c, n=6) and crossbreed control (C_c, n=6). All calves were managed identically, being housed individually, with the experimental groups receiving 2 ml of multi-strain probiotic (Enterococcus faecium and Lactobacillus plantarum) per day. Blood sampling and growth performance were assessed at 1st, 14th and 28th days of the trial. Probiotic supplementation had no significant effects on hematologic profile (P>0.05). However, serum blood glucose and total cholesterol were significantly lowered (P<0.05) in the probiotic treated groups. Multistrain probiotic administration had significant effects on calves (P<0.05), improving the weight gain of both dairy and crossbreed groups (E_d and E_c) compared with control groups (C_d and C_c), at the end of the trial. Current results suggest that multi-strain probiotics support calves early-stage development.

Key words: calves, *E. faecium*, health, *L. plantarum*, performance.

INTRODUCTION

In intensive dairy and beef farming, neonatal diarrhoea affects calves world-wide, generating significant production and financial losses (Medrano-Galarza et al., 2018), that are reflected in high rates of morbidity (up to 35%) and mortality (up to 10%) (Urie et al., 2018). Moreover, the calves that overcome the diarrhoeic episodes, present impaired growth and development, negatively affecting their productivity at maturity (Aly et al., 2016). Therefore, new strategies to improve unweaned calves gastrointestinal health are necessary for mitigating their susceptibility to enteric infections.

Supplementing multi-strain probiotics has been reported to improve early development in calf growth rates (Zhang et al., 2016; Guo et al., 2022) and the immune response (Liang et al., 2020), which reduce the pathogenic risks at farm level (Alayande et al., 2020). Probiotic feed supplementation modulates the gastrointestinal development (Sahu et al., 2019) and the immune system response of neonatal and suckling calves (Arshad et al., 2021) during the first weeks of life. Probiotic such as *Enterococcus faecium* had considerable effects on rumen development and growth rates (Arne and Ilgaza, 2021) when supplemented to beef x dairy crossbreed calves. Furthermore, lactic acid bacteria such as *Lactobacillus plantarum,* was widely studied as probiotic in calves nutrition, in order to improve growth performance, feed efficiency and nutrient availability. However, the efficiency of combining both probiotics has rarely been investigated in suckling calves. Therefore, it is essential to determine the application of multistrain probiotic (Wang et al., 2018) on dairy and crossbreed calves' growth rates and health status.

The aim of the current study was to evaluate the effects of multi-strain probiotic administration on dairy and crossbreed calves' growth performances and health status.

MATERIALS AND METHODS

The experimental design and protocol used were in accordance with the EU Directive

2010/63/EU and the Romanian Law on Experimental Animal Protection.

Calves, husbandry and experimental conditions

Twenty-four dairy and twelve crossbreed calves were assigned (half-sib groups, balanced for age and sex) in four experimental groups (Table 1). The calves farm management was identical, being housed in individual hutches (2.0 x 1.2 x 1.4 m), on deep straw bedding. At birth all calves were fed with 4 kg of colostrum per day, in two equal meals at 12 hours intervals, during their first 3 days of life. Suckling calves received a diet consisting out of 6 kg of milk, into two equal meals per day (at 6:00 AM and 6:00 PM). After the 10th day of life, all calves had *ad libidum* access to water, starter concentrates (18.2% CP) and alfalfa hay until the age of 90 day, when weaning took place.

Table1. Experimental design and dietary treatments

Group	Dietary treatments
C_d	Negative control group - fed with milk (n=12);
E_d	Experimental dairy - fed with milk + Pro (n=12);
C_c	Negative control group - fed with milk (n=6);
E_c	Experimental crossbreed - fed with milk + Pro (n=6).

The probiotics administration was performed daily during the first 28th days of the trial, in drinking water: 1 x 10^6 CFU/ml *Enterococcus faecium* + 1 x 10^8 CFU/ml *Lactobacillus plantarum* (commercial probiotic).

Growth parameters and health

The calves body weights were recorded periodically using a weighing scale platform, on days 0, 14 and 28 of the trial, in order to calculate the average daily gain (first 14th days, last 14th days and the overall trial duration of 28 days).

Before morning feed, blood samples were collected from the jugular vein in all calves, on days 0, 14 and 28, using vacutainer tubes with K_3EDTA and clot activator (Kima®, Italy). Complete blood count (CBC) determinations were performed using an automatic analyser (Diatron, Abacus Junior Vet, Hungary). The haematological screened parameters were: red blood cells count (RBC), haemoglobin concentration (HGB), total white blood cells count (WBC), haematocrit (HCT), mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC).

Calves serum blood was separated by centrifugation (3000 rpm, 15 min, SIGMA), collected in 1.5 ml Eppendorf tubes and stored at -20°C, until further analysis. Metabolic serum profile determination used a semi-automatic analyser (StarDustMC15, DiaSys, Spain), recording: glucose (Glu), total cholesterol (TCho), total protein (TPro), albumin (Alb), aspartate- aminotransferase (TGO), gamma glutamil-transferase (GGT), alkaline phosphatase (PAL), calcium (Ca), magnesium (Mg) and phosphorus (P).

During the 28th days of the trial, faecal scoring for fluidity and consistency, and the calves scoured days severity and treatment were recorded daily each morning (10:00 AM). Faecal scores (Table 2) based on a five-point scale were recorded daily using the procedure of (Yao et al., 2020).

Table 2. Experimental calves growth parameters

Score	Degrees and appearance
1	Thick in consistency, normal;
2	Thin but not watery, normal;
3	Slight watery, abnormal;
4	Watery with dark colouring, abnormal;
5	Watery with light colouring, abnormal.

Statistical analyses

Data were analysed employing the ANOVA procedure with SPSS software (version 20 Inc. Chicago, IL, USA). When comparing treatments means, Post hoc Tukey's multiple range test was carried out to assess any significant differences for the measured parameters. Differences were considered significant at a level of P≤0.05, while the tendency was set at P≤0.10.

RESULTS AND DISCUSSIONS

Results for calf body weight gain (BWG), average daily gain (ADG) and faecal score are presented in Table 3. BWG of suckling calves that received multistrain probiotics treatment had significantly higher values (P≤0.05) at the end of the trial, compared with the control groups. Moreover, dairy calves BWG showed a tendency (P=0.090) towards significance, starting the first 14th day of the trial, compared with the experimental groups. Furthermore, probiotics influenced positively ($P \leq 0.05$) the ADG of both dairy and crossbreed calves groups (E_d and E_c), compared with the control groups. The mean faecal scores registered were significantly lowered ($P \leq 0.05$) by the dietary treatments, starting from the 14^{th} day of the trial. Similar to our findings, recent studies regarding dietary supplementation of *Lactobacillus plantarum* reported an improvement on calves growth rates (Jiang et al., 2020) and faecal scores (Casper et al., 2021). Moreover, by using multi-strain probiotic Guo et al. (2022) found an improvement in dairy calves ADGs and lower the faecal scores. Preweaning is known to be a particular difficult stage for the growth and development of the gastrointestinal tractus in dairy breeds. Therefore, early weaning strategies are often adopted in the intensive dairy cattle industry, in order to support the production and reproductive performance of heifers and to increase the farm economic returns. Calves probiotics supplementation had as effect an improvement of the weaning weight, with higher values of ADG, during the 28^{th} days of trial. The calves higher growth rates in the experimental groups could be attributed to the gastric modulative role of the probiotics, that might had established a balanced rate between the enteric benefic and pathogenic populations, enhancing nutrient availability and feeding efficiency.

Table 3. Body weight gains, average daily gains and faecal scores in dairy and crossbreed calves groups

Parameters		Dairy calves' groups				Crossbreed calves' groups			
		C_d	E_d	SEM	P-value	Cc	Ec	SEM	P-value
BWG	1^{st} day	54.98	60.63	3.769	0.300	54.27	66.87	4.365	0.069
	14^{th} day	63.00	72.34	3.719	0.090	62.80	76.23	5.6689	0.126
	28^{th} day	72.10^b	80.96^a	3.793	0.038	73.3^b	92.17^a	5.823	0.045
ADG	first14^{th} days	0.574^b	0.837^a	0.048	0.001	0.610^b	0.699^a	0.161	0.799
	last 14^{th} days	0.650^b	0.830^a	0.060	0.047	0.750^b	1.138^a	0.130	0.061
	overall trial	0.612^b	0.833^a	0.033	<0.001	0.680^b	0.904^a	0.068	0.042
Faecal score	first 14^{th} days	1.964	1.607	0.085	0.007	1.833	1.631	0.110	0.222
	last 14^{th} days	1.872^a	1.560^b	0.124	0.037	1.952^a	1.393^b	0.093	0.002
	overall trial	1.896^a	1.583^b	0.084	0.007	1.893^a	1.512^b	0.089	0.013

Different superscripts within the same row are different

Diet multi-strain probiotics supplementation had no effects on the calves CBC levels ($P>0.05$). Furthermore, all experimental calves had similar values on CBC parameters, that fell in between the physiological range for the species and age category, as previously published by the Merck Veterinary Manual (2014) and Weiss and Wardrop (2011).

Serum glucose concentration (Glu) was significantly lowered ($P \leq 0.05$) during 14^{th} to 28^{th} day on the probiotics treated groups (E_d and E_c), compared with the control groups (C_d and C_c). Moreover, lowest mean Glu values were observed in the E_d group (59.42 mg/dl), compared with all experimental groups. Calves serum total cholesterol concertation (E_d and E_c) was significantly lower ($P \leq 0.05$) in the probiotic supplementation groups at the end of the trial. However, probiotic supplementation had no effect ($P>0.05$) on serum total protein and albumin concentrations of $E_{d,}$ E_c, C_d and C_c groups. No differences ($P>0.05$) were observed on calves serum enzymatic profile (GOT, GGT and PAL).

Probiotic supplementation might have effects on the nutrient availability during suckling and preweaning stages (Salazar et al., 2019). Lower plasma glucose levels are often a consequence of higher levels of insulin secretion that enhances the energetic metabolism facilitating cellular absorption (Stahel et al., 2016). Our findings are in line with Ilgaza and Arne (2020), who found that dietary supplementation of *E. faecium* decreased the calves serum glucose.

The results for serum TCho values in the current paper are similar to those obtained by Jiang et al. (2020), which observed that calves treated with lactic acid bacteria had lower TCho concentrations. Apart from that, lactic acid bacteria such as *L. plantarum* display the ability to absorption the serum TCho into their own cells (Gupta et al., 2020), having implications in the TCho metabolism regulation (Albano et al., 2018).

Table 4. Calves blood constituents and serum metabolic profiles

Blood parameters		Dairy calves				Crossbreed calves			
		C_d	E_d	SEM	P-value	C_c	E_c	SEM	P-value
Complete blood count									
	1st day	9.98	8.32	1.101	0.303	9.25	8.11	0.511	0.154
WBC	14th day	9.23	9.42	0.711	0.851	9.21	6.47	0.924	0.069
	28th day	9.51	8.74	0.613	0.387	9.14	8.00	0.762	0.324
	1st day	8.75	9.45	0.378	0.213	8.99	8.79	0.785	0.866
RBC	14th day	9.08	9.81	0.308	0.115	9.39	9.87	0.602	0.588
	28th day	9.06	9.15	0.354	0.854	8.19	9.31	0.990	0.445
	1st day	24.00	27.18	1.532	0.164	25.45	25.97	2.256	0.876
HCT	14th day	26.72	27.61	1.138	0.586	26.52	28.37	1.235	0.321
	28th day	25.36	28.70	1.407	0.115	24.57	27.90	2.240	0.323
	1st day	8.75	9.35	0.423	0.333	8.44	9.36	0.552	0.273
HGB	14th day	9.51	8.98	0.398	0.356	8.90	9.86	0.474	0.190
	28th day	8.34	9.84	0.346	0.086	8.80	10.00	0.406	0.070
	1st day	29.63	29.34	1.288	0.893	28.40	30.20	2.302	0.595
MCV	14th day	29.50	28.13	0.940	0.318	28.60	29.00	1.371	0.842
	28th day	27.75	28.75	1.048	0.511	28.40	28.40	1.749	1.000
	1st day	32.91	34.80	0.851	0.139	33.48	36.84	2.288	0.329
MCHC	14th day	33.49	34.54	0.466	0.134	33.44	34.76	0.672	0.202
	28th day	35.84	35.03	1.142	0.623	36.82	36.52	2.572	0.936
Serum biochemical profile									
	1st day	79.58	81.40	4.556	0.785	84.94	84.36	3.781	0.917
Glu	14th day	86.60^{a}	70.81^{b}	3.410	0.011	88.20^{a}	63.89^{b}	3.729	0.002
	28th day	79.81^{a}	59.42^{b}	2.362	<0.001	78.56^{a}	62.76^{b}	3.453	0.120
	1st day	140.20	138.40	5.224	0.813	144.27	145.25	4.116	0.870
TCho	14th day	141.94	148.90	5.325	0.383	144.38	149.15	7.161	0.650
	28th day	148.43^{a}	130.66^{b}	3.95	0.013	151.75^{a}	138.67^{b}	3.53	0.031
	1st day	5.59	5.37	0.301	0.616	5.52	5.54	0.307	0.975
TPro	14th day	5.65	5.78	0.169	0.624	5.96	5.82	0.128	0.461
	28th day	5.80	5.78	0.188	0.930	6.02	5.95	0.212	0.796
	1st day	2.45	2.71	0.145	0.234	2.82	2.60	0.137	0.282
Alb	14th day	2.70	2.59	0.185	0.702	2.79	2.61	0.181	0.493
	28th day	2.60	2.28	0.136	0.132	2.40	2.87	0.184	0.108
	1st day	10.60	10.79	0.811	0.866	9.72	11.08	0.772	0.247
GGT	14th day	11.24	11.52	0.671	0.467	11.54	10.70	0.758	0.454
	28th day	11.67	12.04	0.711	0.734	11.35	11.56	0.780	0.850
	1st day	18.78	21.17	1.744	0.360	19.74	19.53	1.771	0.936
GOT	14th day	20.01	21.37	1.399	0.512	19.52	20.62	1.185	0.531
	28th day	20.62	20.56	0.775	0.962	20.62	19.70	0.858	0.465
	1st day	33.50	33.45	3.240	0.992	30.83	36.56	3.072	0.224
PAL	14th day	35.55	36.36	4.377	0.899	38.76	39.65	2.169	0.852
	28th day	37.79	34.53	3.283	0.503	38.02	39.71	3.144	0.715
	1st day	8.72	9.96	0.185	0.307	9.21	9.24	0.213	0.343
CA	14th day	9.77	9.56	0.134	0.663	10.67	10.87	0.080	0.220
	28th day	9.30	9.90	0.089	0.441	9.90	9.46	0.120	0.330
	1st day	2.91	2.63	0.440	0.082	2.88	2.58	0.345	0.949
MG	14th day	2.55	2.46	0.338	0.669	2.43	2.96	0.645	0.528
	28th day	2.58	2.68	0.428	0.356	2.67	2.84	0.467	0.530
	1st day	7.08	6.56	0.970	0.346	6.41	6.48	0.957	0.344
P	14th day	7.18	6.94	0.669	0.809	6.22	6.57	0.661	0.719
	28th day	6.64	6.97	0.708	0.750	5.90	7.00	0.466	0.134

CONCLUSIONS

Probiotics supplementation had no significant effects on hematologic profile of calves. However, serum blood glucose and total cholesterol levels were significantly lower in the probiotics treated groups. Multi-strain probiotic administration had significant effects on calves, improving the weight gain of both dairy and crossbreed groups, compared with the controls. Current results suggest that multi-strain probiotics support calves early-stage development. Early calves ruminal development and health status have a direct influence on the rearing management, lowering the age of weaning and costs related with veterinary treatments. Further studies are needed in order to investigate the mechanisms through which the multi-strain probiotic dietary supplement affect the energetic serum profile in pre-weaned calves.

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