EFFECTS OF A COMBINED PRE- AND PROBIOTICS PRODUCT ON DIARRHOEA PATTERNS AND PERFORMANCE OF EARLY WEANED CALVES

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Summary. The interest in probiotics, prebiotics and other biological additives has greatly increased following the banning of antibiotic growth promoters by the European Union and consumer awareness of „safe and functional foods". This experiment was designed to provide experimental data on the effects of feeding combined pre- and probiotics product to early weaned dairy calves.

Twenty four Lithuanian Black-and-White calves were weaned at 6 days of age and were randomly assigned to two treatments (12 experimental calves and 12 control calves). The calves were fed either a diet supplemented with a combined pre- and probiotics product (*Enterococcus faecium* M74 with a non-digestible oligosaccharide (NDO) or a control diet for 9 weeks.

During the entire trial each calf was fed milk replacer twice a day presented in plastic buckets and and the pre- and probiotics supplement for experimental calves was administered daily from experimental day 1 to 63 (from 6 to 68 days of age). Starting the first week of the experiment, calf starter (compound feed), hay and fresh water were supplied *ad libitum.*

Combined pre- and probiotics product supplementation markedly lowered occurrence of post weaning diarrhoea and severity of the diarrhoea. In calves on pre-and probiotics diet the percentage of calves with diarrhoea reduced from 65 % to 25 %. Calves given the combined pre-and probiotics product had the forage and total DM intakes that were higher than those fed control diet, without any additive. During the 63 days experimental period, average daily gain and feed conversion rate were improved by 15.3 % ($P<0.05$) and by 12.8 % ($P<0.05$) respectively in the pre- and probiotics treated group.

Key words: calves, pre- and probiotics product, feed intake, growth rate, diarrhoea.

ANKSTI ATJUNKYTŲ VERŠELIŲ VIDURIAVIMO MAŽINIMAS IR AUGIMO GERINIMAS Į PIENO PAKAITALĄ PRIDEDANT PRE- IR PROBIOTINIO PRODUKTŲ JUNGINIO

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Santrauka. Europos Sąjungos šalyse, uždraudus į pašarus dėti antibiotikus, padidėjo dėmesys probiotikams, prebiotikams ir kitiems biologiniams priedams, gerinantiems gyvūnų sveikatą, augimą ir užtikrinantiems „saugų ir funkcionalų maistą". Šis eksperimentas atliktas norint nustatyti pre- ir probiotinio produktų junginio įtaką anksti atjunkytų veršelių viduriavimui ir jų augimo intensyvumui.

Bandymams paimti šešių amžiaus dienų atjunkyti 24 Lietuvos juodmargių veislės veršeliai, atsitiktinai suskirstyti į dvi grupes (12 kontrolinėje grupėje ir 12 tiriamojoje). Devynias savaites trukusio bandymo metu abi veršelių grupės du kartus per dieną buvo girdomos vienodu pieno pakaitalu, bet į tiriamosios grupės veršelių pieno pakaitalą kasdien buvo dedama po 10 gramų pre- ir probiotinio produktų junginio (probiotinės bakterijos *Enterococcus faecium* M74 derinys su nevirškinamais oligosacharidais). Nuo pat bandymų pradžios veršeliams iki soties buvo duodama starterinio kombinuotojo pašaro, šieno ir vandens. Pre- ir probiotinio produkto priedas ženkliai sumažino viduriuojančių veršelių skaičių ir palengvino viduriavimą. Viduriuojančių veršelių, gavusių pre- ir probiotinio produkto, sumažėjo nuo 65 iki 25 proc. Tiriamosios grupės veršeliai pašarų sausųjų medžiagų suėdė daugiau, negu kontrolinės grupės veršeliai. Per visą 63 dienų bandymo laikotarpį veršelių, gavusių pre- ir probiotinio produktų junginio, vidutinis paros priesvoris buvo didesnis 15,3 proc. ($p<0,05$), o pašarų sąnaudos 1 kg priesvorio – 12,8 proc. ($p<0.05$) mažesnės, negu kontrolinių veršelių.

Raktažodžiai: veršeliai, pre- ir probiotinio produktų junginys, viduriavimas, pašarų sąnaudos.

Introduction. The first months of life are very critical for calves with regard to realizing final goal i.e. to achieve well-developed heifer or bull. The milk-feeding period for calves represents the time of life when dairy animals are subject to the greatest number of health problems. Calves are often affected by diarrhoea and respiratory disease. Many factors could trigger off the high incidence of intestinal and respiratory disease in calves. After birth, calves are separated from their mothers, preventing the calf from picking up the protective gut flora from its mother (Fuller, 1989). Furthermore, young animals are faced with major stress events like transportation, dietary changes and exposure to a variety of infectious agents. Calves consume less milk (Loerch and Fluharty, 1999) and predisposed to loss of barrier function of the gut (Soderholm and Perdue, 2001) and the protective potential of the microbial gut flora tends to decrease (Hooper et al., 2001; Fuller, 1989) indicate that during stress events, the trend is for the protective lactobacilli to decrease and for coliforms to increase. When homeostatic control is disturbed, chronic inflammation, diarrhoea and disease may occur.

Nowadays, the emergence of antibiotic resistance in the human commensal bacteria has raised concerns about the impact of antimicrobial compounds for agriculture use and has accelerated the search for alternative nutritional strategies, such as the addition of probiotics and prebiotics (Verstegen et al., 2002). With the EU ban on the use of antibiotic feed additives the use of probiotics and prebiotics for the establishing a protective flora in calves is promising. Probiotics are certainly another group of growth enhancing additives (Ziggers, 2001). Probiotic supplementation of this microflora is intended to stimulate the establishment of beneficial bacteria in the gut and to retard the proliferation of pathogens. Through stabilization of desirable gastrointestinal microflora probiotics have a positive effect on the function of digestive tract.

Various probiotics bacterial cultures have been examined and promoted for use in calves and piglets diets. Probiotics have been noted in many studies and has shown that positive effect(s) of probiotic may vary according to the culture of probiotic (Denev, 1996; Cruywagen et al., 1996, Timmerman et al., 2005). There are currently a number of probiotic preparations available for application to calves. Research with probiotics added to diets of young calves have been equivocal. In some experiments (Donovan et al., 2002; Khuntia and Chaudhary, 2002), improvements in animal health and performance have been reported, in others (Cruywagen et al., 1996), no effect of the inclusion of probiotics has been found. It is probable that effects are dependent on what kind or strain of specific bacteria was used and dosage of product and on environmental and feeding conditions. However, the effectiveness of using probiotc depends, among others, on the composition of feeds, especially content of nutritional stimulants of probiotic bacteria growth (Fleige et al., 2007). Among them prebiotics, mainly nondigestible oligosaccharides seems to be the most promising.

Prebiotics are non-digestible food ingredients that stimulate the growth and activity of bacteria in the digestive system which are beneficial to the health of the body. Generally, however, it is assumed that a prebiotic should increase the number and activity of bifidobacteria and lactic acid bacteria and have shown promise in young calves (Kaufhold et al., 2000). The prebiotics promote only the species-specific probiotic microflora, while one of the effects of the *Enterococcus faecium* is the swift development of an intestinal environment that is favourable to exactly the same microorganisms. This approach may be utilised as a means of performance enhancement and for reducing disease incidence in livestock.

The aim of this experiment was to investigate the effect of a combined pre-and probiotics product (*Enterococcus faecium* M74 with a non-digestible oligosaccharide (NDO) on diarrhoea patterns and performance of early weaned calves.

Materials and methods. As recommended by the Scientific Committee on Animal Nutrition (SCAN), the efficacy of pre-and probiotics product was assessed according to the Directive No. 87/153/EEC. The feed mixture, milk or/and milk replacers not contain medicated additives. The experiment was arranged and conducted in due form using animal number in groups and number of groups that are satisfactory for establishing the minimum claimed response. The experiment was conducted under good hygienic conditions. The feeding trial was performed regarding the Lithuanian animal care, management and operation legislation (No 8-500, 28 November 1997, no 108).

Experimental conditions. The trial was carried out in calf house with separated pens, each of which was equipped with feeding trough and watering trough as required for calves. All pens were located in the same calf house and the calves were randomly allocated. The total area per pen was 8.5 m^2. The calf house was equipped with controlled ventilation and the bedding in the pens was chopped straw. Manure was removed daily and chopped straw was given to all pens again. Temperature, air humidity and concentration of ammonia in the calf house were monitored. Average temperature was 14.5±2.0^{0}C, relative humidity was 75.0±5.0% and ammonia concentration averaged at 6.5 mg/m^3.

Experimental animals. Lithuanian Black-and-White calves were taken from their dams at 6 days of age and 24 calves on the basis of initial weight and sex were selected for the trial. All calves were marked with earmarks after birth. The actual trial period started at weaning (day 0) when calves were 6 days old and stopped 63 days later. At weaning the calves were divided into two analogous groups according to weight and each group was moved to a pen in the calf house. The gender of the calves was also considered. There were 12 calves in the pre-and probiotic product (experimental) group and 12 calves in the control group. Each group consisted of 6 male calves and 6 female calves. The initial average weight of the calves in the two group was identical, i.e. 41 kg. Each group was further divided into 2 groups of six calves and placed into two separate pens.

The health status of the calves was monitored daily with particular attention paid to the occurrence of diarrhoea. All calves were given a diarrhoea score according to: 0 = firm, no signs of diarrhoea, 1= soft, slightly loose faecal consistency and 2 = liquid, very loose faecal consistency. For each calf the daily scores and the number of days with liquid faeces (score 2) was summed into an index of the severity of the diarrhoea.

Feeds and feeding. During the entire trial the calves were fed twice a day with milk replacer presented in plastic buckets. The replacer was reconstituted to 12% dry matter as recommended by the manufacturer. Starting the first week of the experiment, calf starter (compound feed), hay and fresh water were supplied *ad libitum* (Table 1).

One and the same milk replacer, calf starter (compound feed) and hay were used for the whole trial. A formulated calf starter contained cereals as the major source of energy and was manufactured by joint-stock company Kedainiu grudai. The calf starter contained no growth promoters or other additives. Hay was made from first cut grass-legume sward. Feeds (milk replacer, calf starter and hay) chemical composition and nutrient content were analysed at the Chemical laboratory of the institute of Animal Science of LVA. Samples of each feed were taken regularly during the experiment and chemical composition of the feeds was determined according to AOAC procedures (1995). The composition and the analyzed nutrient content of offered feeds are provided in Table 2.

The milk replacer, calf starter and hay used to feed the experimental as well as the control calves had the same nutritional value and quality. Calves in pre-and probiotic group were given daily 10 g product per calf (120 g per group) with the morning and evening milk replacer during all experiment. The control group received any additives.

Table 1. **Feeding scheme**

Calves age, weeks	Intended live weight on the end of period	Feed per calf per day, kg		
		Whole milk	Starter	Hay
1		6	0.04	-
2		6	0.12	-
3		6	0.25	-
4	51	6	0.36	0.1
5		6	0.52	0.2
6		6	0.72	0.3
7		6	1.00	0.35
8		6	1.30	0.40
9	72	6	1.50	0.45
Total per 9 weeks		378	31.22	12.6

Table 2. **The composition and the analysed nutrient content in the diet fed to the calves**

Composition %	Calf starter	Milk replacer	Hay
Barley	42.5		
Wheat	20.0		
Triticale	6.0		
Soybean meal	18.5		
Rapeseed cake	8.2		
Dicalcium phosphate	2.5		
Sodium chlorine	0.3		
Mineral and vitamin supplement	2.0*	**	
Analyzed content:			
Dry matter %	87.5	94.0	82.7
Crude fibre, g kg^{-1}	46.0	3.0	251.8
Crude protein, g kg^{-1}	192.0	220.0	128.7
Fat, g kg^{-1}	26.0	150.0	18.9
Calcium, g kg^{-1}	11.1	8.0	6.7
Phosphorus, g kg^{-1}	12.2	8.0	3.3
Zinc, g kg^{-1}	-		
Lysine, g kg^{-1}	9.1		
Methionine + cystine, g kg^{-1}	5.4		
Tryptophan, g kg^{-1}	2.2		
Threonine, g kg^{-1}	6.2		

*Provided (pp kg^{-1}): iron 25.0, manganese 100.0, cobalt 2.5, iodine 0.0, selenium 0.2. Vitamin A 20000 IU kg^{-1}, vitamin D_3 4000 IU kg^{-1}, vitamin E 40 IU kg^{-1}.

**Provided: Vitamin A 50000 IU kg^{-1}, vitamin D_3 5000 IU kg^{-1}, vitamin E 150 IU kg^{-1}.

Weighing of the calves. All calves were weighed at 6 days of age (at weaning), at 16 days of age, at 26 days of age, at 48 days of age and at 69 days of age.

Monitoring of health. As a routine on the farm all occurrences and treatments of disease and injuries were noted individually.

Statistical evaluation. The data was analyzed as a randomized complete block with repeated measurements (the same calf was weighed on different time periods). The feed additive treatment was the main factor. For the data on calf weight, the calf was considered the experimental unit. The data on feed intake, the pen with 6 or 12 calves was considered the experimental unit, as feed intake was determined on a group of calves, not individual calves. Results were analysed using GLM of SAS.

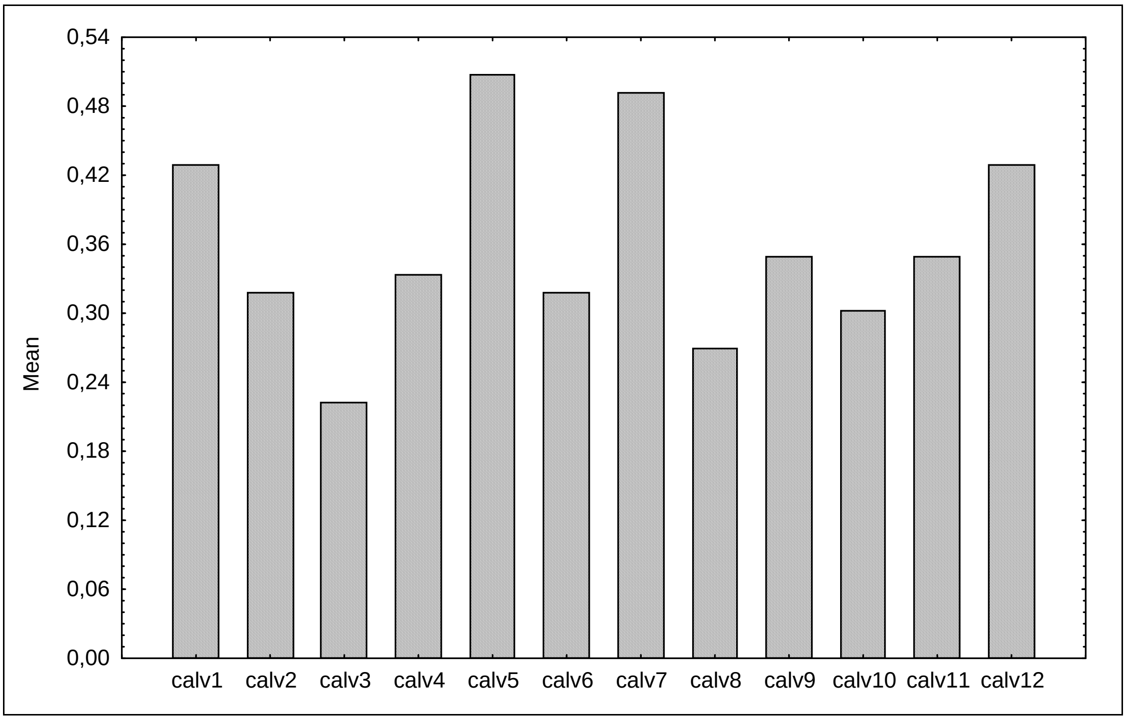

Figure 1. **The diarrhoea score calf^{-1}day^{-1}during experimental period** (63 days) **of the control calves**

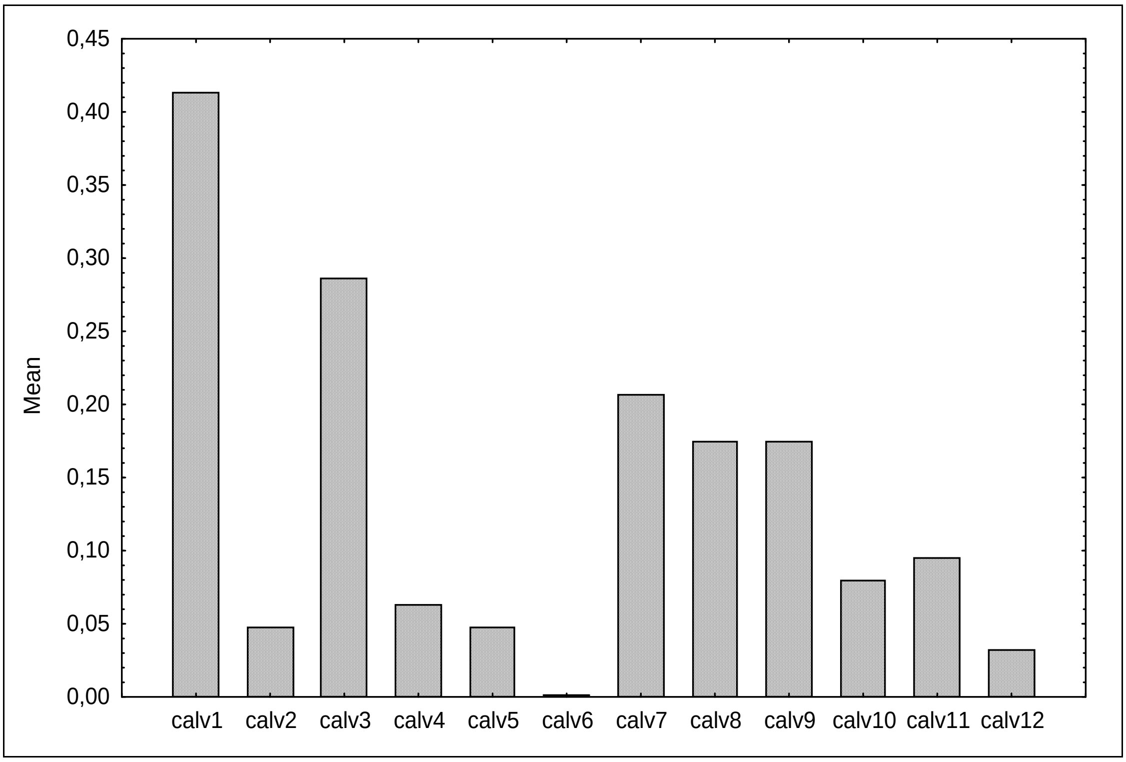

Figure 2. **The diarrhoea score calf^{-1}day^{-1}during experimental period** (63 days) **of the calves given pre-and probiotic product**

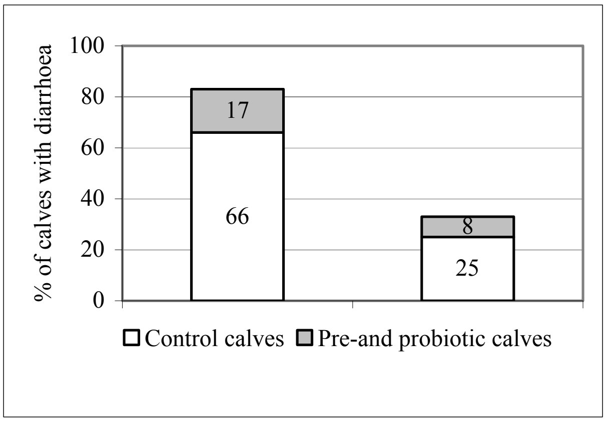

Figure 3. **Effects of pre-and probiotic product supplementation on the percentage of calves with diarrhoea.** The percentage of calves with diarrhoea for one day (white plus blank column) and for two or more days (white column).

Results and discussion. *Diarrhoea incidence*. No calves died during the experiment. The diarrhoea was seen during the first 23 days after weaning for calves fed untreated diet, but only during the first 8 days after weaning for calves fed the diet with pre-and probiotic product. The diarrhoea score calf^{-1}day^{-1} was markedly lower for the experimental calves as compared with that of the untreated diet calves (Figure1 and Figure 2). In four experiments (Timmerman et al., 2005) was found that probiotic treatment systemically reduced the occurrence of diarrhoea and probiotic preparation significantly depressed the incidence and duration of mild and total diarrhoea.

The dietary pre-and probiotic product content as shown in Figure 3 influenced the percentage of the calves that had diarrhoea. The percentage of calves having diarrhoea was reduced from about 66 % to 25 % among the calves fed pre-and probiotic product diet. Prebiotics and probiotics have been proposed to manipulate the bacterial flora of the intestinal tract of animals to potentially reduce the incidence of diseases (Bohmer et al., 2005), and they have direct effects on immune responses (Macfarlane and Cummings, 1999; Pie et al., 2007).

Table 3. **Body weight, average daily gains, feed intake and feed conversion rate of calves supplemented pre- and probiotic product**

	Supplement pre-and probiotic product		$LSD_{0.05}$	P
	0	10 g per calf/day		
Number of calves	12	12		
Body weight, kg per calves				
At weaning(6 days of age)	41.0	40.9	2.92	ns
At 16 days of age	44.3	45.4	3.04	ns
At 26 days of age	48.9	52.5	4.24	ns
At 48 days of age	61.7	66.7	4.83	0.05
At 69 days of age	78.3	84.0	6.02	ns
Daily gain, g per calves				
6 to 15 days	325	450	141.1	ns
16 to 25 days	467	708	201.8	0.05
26 to 47 days	579	644	63.3	0.05
48 to 68 days	790	820	165.1	ns
6 to 68 days	593	684	74.9	0.05
Feed intake, kg DM per calf				
6 to 15 days	6.6	6.6	0.00	ns
16 to 25 days	7.9	8.6	3.88	ns
26 to 47 days	23.7	23.8	2.03	ns
48 to 68 days	31.4	31.4	11.37	ns
6 to 68 days	69.6	70.4	5.84	ns
Feed conversion rate, kg DM feed per kg gain				
6 to 15 days	2.04	1.49	0.89	ns
16 to 25 days	1.70	1.24	0.13	0.05
26 to 47 days	1.86	1.68	0.00	0.01
48 to 68 days	1.89	1.81	1.52	ns
6 to 68 days	1.87	1.63	0.38	ns

Weight and weight gains of the calves. The average weight of the calves in control and experimental groups at start (6 day age) was equable, i.e. 41.0 kg in the control group and 40.9 kg in the experimental group. Evaluation of experiment in the calves rearing have shown that pre- and probiotic product caused a 7.2 % i.e. 5.7 kg increase in body weight at 69 days of age, i.e. after 63 days of the all trial period. During the 42 days of the trial, i.e. at 48 days of age, the difference was 8.1%, i.e. 5.0 kg (P<0.05). After the 20 days of the experiment, i.e. at 26 days of age, and after 10 days of the experiment, i.e. at 16 days of age, the weight of the experimental calves were by 7.4 % (3.6 kg) and by 2.4 % (1.1 kg) higher in comparison with the control calves (Table3). Similar observations were made by Cruywagen et al. (1996) who showed that administration of a monostrain probiotic (L. acidophilus) resulted in maintenance of initial BW during wk 0 to 2 vs. a 1.6-kg weight loss in nontreated animals.

The average weight gain of calves given in-feed pre- and probiotic product additive was 15.3 % (P<0.05) of that in the control group for the all trial (63 days) period (from start of trial 6 days of age and to end of trial 68 days of age). The experimental calves also showed a significant higher growth during period at 26 to 47 days (10.9%, P<0.05) and period at 16 to 25 days (51.1%, P<0.05)

Table 3 results shown that with pre-and probiotic product it is possible to improve daily weight gain of the calves. The daily weight gain of the calves fed pre-and probiotic product was significantly higher than that of control calves in period at 16 to 25 days (P<0.05), at 26 to 40 days (P<0.05) and over total trial at 6 to 68 days (P<0.05). This outcome agrees with that of other studies in calves (Alves et al., 2000) when average values of body weight, daily gain, feed efficiency, weight and dressing percentage of carcass were significantly higher for calves fed whole milk with probiotic but in other studies (Aydin et al., 2008), no probiotic-induced reduction of the weight and weight gain in various stages of the growth of the calves and feed efficiency ratio was observed.

Feed intake and conversion rate. Feed intake in each of the four pens was monitored separately. The milk replacer intake was identical for all 24 calves in both groups, i.e. 0.658 kg DM of milk replacer per calf and day. No major differences were found for the groups fed pre-and probiotic product supplement or pre-and probiotic product free. The total DM intake was numerically higher in the experimental group in period at 16 to 25 days and in all trial period, at 6 to 68 days (Table 3). The values of feed conversion rate clearly demonstrate the effect of use of pre-and probiotic product at first two decades after weaning. For the trial period from 6 to 15 days and for the trial period from 16 to 25 days the calves in experimental group consumed respectively 0.55 kg (27.0 %) and 0.46 kg (27.0 %, P<0.05) less feed (DM) per kg of weight gain than the calves in the control group In the period at 26 to 47 days the feed conversion rate was significantly (P<0.05, P<0.01) improved when pre-and probiotic product supplement was used. For the whole trial (from 6 to 68 days) feed conversion rate was improved by 0.24 kg per kg of weight (12.8 %).Administration of probiotic strains separately and in combination was significantly improved feed intake, feed conversion rate, daily weight gain and total body weight in chicken, piglets and calves (Verstegen and Williams, 2002. Li et al., 2006; Samli et al, 2007)

Conclusions.

1. Combined pre-and probiotic product supplementation markedly lowered occurrence of post weaning diarrhoea and severity of diarrhoea for early-weaned calves. In calves on pre-and probiotics diet the percentage of calves with diarrhoea reduced from 65 % to 25 %.

2. Calves fed the combined pre-and probiotic product were higher in average body weight by 7.2 % and in average daily weight gain by 15.3 %

3. The results of this trial have shown that the combined pre-and probiotic product supplemented to milk replacer not caused a markedly additional increase in feed dry matter intake but caused a 12.8 % improvement in the feed conversion ratio.

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