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Effects of probiotics on growth performance in young calves: A meta-analysis of randomized controlled trials

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ABSTRACT

Growth of calves during their first few weeks of life is one of the most important factors affecting their performance during subsequent rearing, and it can be modified by disease, especially gastrointestinal infections. Use of lactic acid bacteria (LAB) is a tool which may maintain the intestinal microbial balance, prevent diarrhea and improve growth. However, a consensus has not been reached as to whether probiotics are effective in improving growth of calves. The objective of this meta-analysis was to assess effects of probiotics on the growth of calves (*i.e.*, body weight gain (BWG), feed efficiency). PubMed and Scopus databases were searched from 1980 to 2010, unrestricted by language. The inclusion criteria were: randomized and controlled experiments using calves less than 5 d of age without apparent disease and with passive immunity, and published in peer reviewed journals. Twenty-one and 14 studies were included to assess probiotic effects on BWG and feed efficiency, respectively. LAB supplementation increased BWG (standardized mean differences (SMD) = 0.22822, 95% confidence interval (CI) 0.1006–0.4638) and improve feed efficiency (SMD = −0.8141, 95% CI −1.2222 to −0.4059), considering the source of heterogeneity and publication biases. Growth of calves was not affected when the LAB was added to whole milk, but beneficial effects occurred when LAB was added to milk replacer. The probiotic effect was not related to the number of LAB strains in the inoculum. The number of calves in the experiments had an impact on the results and conclusions. Probiotics may be an alternative to the antibiotics commonly used as growth promoters in calves.

1. Introduction

Lactic acid bacteria (LAB) are in the normal intestinal microbiota of animals and humans (Schneider et al., 2004; Soto et al., 2010), and have been identified as controllers of pathogens such as *Salmonella* spp. (Gill et al., 2001) and *Escherichia coli* (Shu and Gill, 2002). These pathogens are the etiologic agent of calf diarrheas during the first weeks of life, and diarrhea is the main cause of morbidity and mortality in the early life of calves (Timmerman et al., 2005).

It is very important to reduce the prevalence of gastrointestinal infections in young calves, because when animals are ill at this stage, their subsequent growth is delayed thereby affecting productivity (Rosmini et al., 2004). The high incidence of

Abbreviations: BWG, body weight gain; CI, confidence interval; I^2, Inconsistency index; LAB, lactic acid bacteria; SMD, standardized mean differences.

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intestinal disease is especially high in intensive rearing systems where exposure to pathogens is increased due to confinement of large numbers of animals in small areas (Callaway et al., 2002).

Use of LAB to reduce pathogenic bacteria in the gut has been termed a probiotic strategy, with an overall goal of promoting colonization of protective bacteria in calves during the first weeks of life to compete with pathogenic bacteria. Additionally, LAB can stimulate development of the immune response against pathogenic bacteria and counteract negative effects of illnesses (Frizzo et al., 2010).

To prevent and control intestinal infections, a current practice is to use antibiotics, a strategy which could increase the emergence and spread of antibiotic-resistant bacteria in meat and dairy products. In addition, residual antibiotics in those foods are unacceptable for human consumption. To overcome these problems, use of LAB has been suggested as a feed additive to promote beneficial effects to the host by favoring the balance of the intestinal microbiota (Abu-Tarboush et al., 1996).

Reports of LAB fed to young calves are inconclusive. Many authors reported beneficial effects of probiotic preparations on animal growth (Abe et al., 1995; Meyer et al., 2001; Timmerman et al., 2005; Frizzo et al., 2008, 2010), while others (Jenny et al., 1991; Higginbotham and Bath, 1993; Abu-Tarboush et al., 1996; Cruywagen et al., 1996; Goncalves et al., 2000) reported no effects.

Systematic review consists of a scientific technique of reviewing available literature using explicit methods to identify, select and critically evaluate studies which are relevant to the stated objective (Faria Filho et al., 2006). A consistent review of studies of probiotics added to calves diets and published to date can be completed using meta-analyses. As the use of probiotics in the diet of young calves may improve their growth performance, the objective of this meta-analysis was to assess effects of probiotic supplementation on growth performance (*e.g.*, body weight gain (BWG), feed efficiency) in young calves.

2. Materials and methods

2.1. Criteria for study selection

The studies included in the meta-analysis were selected based on the criteria: randomized and controlled experiments, studies which used calves <10 d of age, without diseases and with passive immunity, and published in peer-reviewed journals. Controlled experiments were defined to include use of a placebo. LAB could be added in whole milk, pasteurized whole milk, or milk replacer and with or without inclusion of a calf starter as a solid diet. All feeds used must have been free of added antibiotics or growth promoters. Studies must have reported BWG and feed efficiency with measure of variance. Reviews, duplicate reports, experiments which used non-identified or non-viable probiotics, and studies which included animals with diseases were excluded. In this meta-analysis, the term "study" was applied to identify each scientific article which can include one or more experiments (each experiment being a controlled experiment to compare a particular combination of probiotic fed and control groups of calves).

2.2. Outcomes and definitions

The impact of LAB supplementation on BWG and feed efficiency were analyzed. Data from each study corresponded to the whole study. In studies which included more than one LAB group, or the same LAB in different diets (*i.e.*, whole milk, milk replacer), each LAB group was compared with the control group separately.

2.3. Data sources

PubMed and Scopus databases were searched from 1980 to 2010 for articles unrestricted by language. Search terms included probiotic* and calves*. Abstracts were assessed and articles that met the *a priori* inclusion criteria were utilized.

2.4. Data extraction

Information on study design, methods (diets), treatments (LAB strains, treatment dose, duration), number of animals, young calf sex and breed, and outcomes, were extracted from each article. Relative to the outcomes of each study, the frequency and methodology applied were analyzed to evaluate the quality of studies. However, no scores were used to exclude studies (Lean et al., 2009).

2.5. Statistical analysis

Statistical analysis used Epidat software version 3.1 (2006). Due to continuous variables being analyzed, results are presented as standardized mean differences (SMD) between the probiotic treatment and controls with 95% confidence intervals. Weights of each study were based on the inverse of the variance. *A priori* subgroup analyses were planned depending on: (1) type of feed (whole milk *versus* milk replacer), (2) study duration (two subgroup analysis restricted were conducted: (a) rearing from 0 to 45 d *versus* 46 to 187 d, and (b) rearing from 0 to 60 d *versus* 61 to 187 d, (3) LAB strain used (with *L.*

acidophilus versus without *L. acidophilus*; with *L. plantarum versus* without *L. plantarum*; with *L. salivarius versus* without *L. salivarius*; with *E. faecium versus* without *E. faecium*; with *Bifidobacterium* spp. *versus* without *Bifidobacterium* spp.; with *L. casei*/*paracasei versus* without *L. casei*/*paracasei*), (4) type of inoculum (mono-strain *versus* multi-strain) and (5) number of calves (≤20 *versus* >20).

Heterogeneity among studies was evaluated using DerSimonian and Laird test (*Q*-statistic) and Inconsistency index (I^2-statistic; Higgins and Thompson, 2002). A classification of I^2 values was used to interpret its magnitude: values about 25%, 50% and 75% were considered as low, medium, and high heterogeneity, respectively (Higgins and Thompson, 2002). If studies were homogeneous, a fixed effects model was used. By contrast, if studies were heterogeneous, a random effect was used. Additionally, to investigate causes of heterogeneity, a meta-regression was conducted. The continuous predictor variables used in the stratified analysis (study duration and number of calves) were included as predictors in a weighted regression with the weight equal to the inverse variance of the result of each study (Dohoo et al., 2003).

Sensitivity analysis was completed to assess the robustness of the meta-analysis results. Sensitivity analyses have also been used to examine effects of studies identified as being aberrant or highly influential in the analysis outcome (Lean et al., 2009). This consists at completing the same analysis (SMD), but dropping one study each iteration.

An adjusted rank correlation test using the Egger method (Egger et al., 1997) and the Begg test (Begg and Mazumdar, 1994) were used to assess publication bias.

3. Results

3.1. Overview of included studies

The literature search yielded 66 scientific papers on probiotics and calves. Twenty one of the 66 screened articles met all inclusion criteria to assess the probiotic effect on BWG, but only 14 articles were included in the evaluation of the probiotic impact on feed efficiency.

Within the studies included to assess the probiotic effect on BWG in young calves, only one study was conducted before 1990, with most between 1991 and 2000 (11) and after 2001 (9). Holstein calves were used in the most of studies (15). The number of calves was variable; on 7 occasions with <20, whereas studies with 21 to 50 and >50 occurred on 9 and 5 occasions, respectively. Thirteen studies were conducted using multistrain probiotics, 5 studies used monostrain probiotics, and the remaining 3 studies used monostrains and multistrains probiotics in the same one. The calves were fed whole milk (8), milk replacer (10), or both in the same trial (3). Studies were conducted for <45 d (7), between 45 and 60 d (9) or >60 d (5; Table 1).

Fourteen studies were included to assess impacts of probiotics on feed efficiency, with 7 conducted between 1991 and 2000, and 7 after 2001. Holstein calves were principally selected (11). The number of calves was variable, with 5 occasions <20, while those of 21 to 50, and >50 were on 8 and 1 occasion, respectively. Nine studies used monostrain probiotics, whereas 2 were conducted using multistrain probiotics and the remaining 3 used monostrain and multistrain probiotics in the same one. The calves were fed whole milk (4), milk replacer (8), or both types of feed in the same study (2). Studies were conducted for <45 d (7), between 45 and 60 d (4) or >60 d (3; Table 1).

3.2. Excluded studies

Of the 66 studies identified at the beginning of the meta-analysis, 34 failed to meet one or more inclusion criteria. Review articles, experiments conducted to assess impacts of probiotics and prebiotics, or experiments to isolate and select strains with potential probiotic activity without any *in vivo* test to study effects on performance of calves were excluded. Eleven experiments which passed initial screening were excluded (Fig. 1) due to lack of statistical information for conducting a meta-analysis (8), experiments that included ill calves or without correct passive immunization (2), and experiments which used non-viable LAB (1).

3.3. Body weight gain

Of the 21 studies that met the inclusion criteria, 36 experiments (1547 calves) that combined calves fed with probiotics and control groups were identified. In the pooled estimate, probiotics increased BWG compared to controls (SMD = 0.22822, 95% CI 0.1006–0.4638) in the pooled standardized mean difference random effect model (Table 2). Heterogeneity occurred among the 36 experiments (*Q*-statistic: P<0.0001; I^2-statistic = 59.95%, 95% CI 52.24–66.41%), with 2 experiments (Frizzo et al., 2010; Isik et al., 2004) responsible for the majority of the heterogeneity. Both of these showed that LAB produce a beneficial effect on growth of young calves. When these two were removed from the analysis, a homogenous group of 34 experiments was analyzed (*Q*-statistic: P=0.084; I^2-statistic: 26.17%, 95% CI 10.89–38.83%), showing that the pooled estimate of BWG was similar (SMD = 0.1913, 95% CI 0.0844–0.2982).

Two subgroup analyses restricted to the type of feed provided to the calves (*i.e.*, whole milk, milk replacer), were conducted. Twelve experiments using whole milk were identified, and probiotics did not impact BWG (SMD = 0.3061, 95% CI −0.0797 to 0.6919; Table 2). Another subgroup analysis restricted to 24 experiments which used milk replacer to calves found an increase in BWG (SMD = 0.2671, 95% CI 0.0624–0.4718; Table 2).

Table 1
Randomized, controlled experiments to study effects of supplementation with probiotics on growth rate and feed efficiency of young calves.

Year	*n*	SMD[a] (95% CI)		Breed	Probiotic	Duration (days)	Feed	Reference
		BWG	Feed efficiency					
1985	45	−0.293 (−0.880 to 0.294)	n.d.	Swedish Red and Withe	*Lactobacillus* spp.	50	Milk replacer	Jonsson and Olsson (1985)
1991	32	0.117 (−0.575 to 0.811)	0.028 (−0.664 to 0.721)	Holstein	*L. acidophilus* and *L. lactis*	42	Milk replacer	Jenny et al. (1991)
1991	32	0.353 (−0.344 to 1.051)	−0.205 (−0.900 to 0.489)	Holstein	*Bacillus subtilis*	42		
1993	50	0.212 (−0.343 to 0.768)	−1.563 (−2.196 to −0.930)	Holstein	*L. acidophilus* and *Streptococcus faecium*	36	Milk replacer	Higginbotham and Bath (1993)
1995	30	1.204 (0.426 to 1.982)	−1.125 (−1.895 to −0.354)	Holstein	*Lactobacillus acidophilus*	56	Milk replacer	Abe et al. (1995)
1995	30	0.610 (−0.310 to 1.532)	−0.412 (−1.323 to 0.497)	Holstein	*Bifidobacterium pseudolongum*	56		
1995	19	1.035 (0.273 to 1.797)	−1.247 (−2.029 to −0.465)	Holstein	*Lactobacillus acidophilus*, *Bacillus thermophilum* and *Enterococcus faecium*	56		
1996	16	0.919 (−0.111 to 1.949)	−0.277 (−1.261 to 0.707)	Holstein	*L. acidophilus* and *L. plantarum*	84	Pasteurized whole milk and milk replacer	Abu-Tarboush et al. (1996)
1996	16	−0.274 (−0.897 to 0.347)	0.000 (−0.619 to 0.619)	Holstein	*L. acidophilus* 27SC	84		
1996	40	0.282 (−0.702 to 1.267)	−0.077 (−1.058 to 0.902)	Holstein	*Lactobacillus acidophilus*	42	Milk replacer	Cruywagen et al. (1996)
1998	38	−0.091 (−0.728 to 0.544)	−0.187 (−0.825 to 0.449)	Holstein	*S. faecium*, *L. acidophilus*, *Saccharomyces cerevisae*, *Bacillus subtilis* and *Aspergillus oryzae*	56	Milk replacer	Higginbotham et al. (1998)
1999	24	−0.122 (−0.922 to 0.678)	n.d.	Holstein × Cebú	*Lactobacillus acidophilus*	56	Whole milk	Chavez et al. (1999)
1999	78	0.109 (−0.334 to 0.553)	n.d.	Not specified	*Bacillus cereus* var. Toyoi	15	Whole milk	Erhard et al. (1999)
1999	20	0.498 (−0.392 to 1.388)	n.d.	Holstein	*Lactobacillus* sp.,	90	Milk replacer	Monti and Tarabla (1999)
1999	20	−0.345 (−1.229 to 0.537)	n.d.	Holstein	*Streptococcus* sp. and Yeast	90		
2000	14	−0.927 (−2.073 to 0.218)	n.d.	Holstein	*Lactobacillus acidophilus*, *Bacillus subtilis*, and *Lactobacillus subtilis*	60	Whole milk Probiotic as powder	Goncalves et al. (2000)
2000	14	0.493 (−0.570 to 1.556)	n.d.	Holstein	*Lactobacillus acidophilus*, *Bacillus subtilis*, *Bifidobacterium bifidum* and *Lactobacillus lactis*	60	Whole milk Probiotic	
2000	14	0.421 (−0.683 to 1.525)	−0.758 (−1.842 to 0.326)	Holstein × Cebu	*Lactobacillus acidophilus*, *Streptococcus faecium* and *Sacharmyces cerevisae*	119	Whole milk	Alves et al. (2000)
2001	30	−0.186 (−1.089 to 0.716)	0.263 (−0.498 to 1.025)	Holstein	*Lactobacillus acidophilus*,	15	Whole milk	Meyer et al. (2001)
2001	30	−0.593 (−1.367 to 0.180)	−3.236 (−4.353 to −2.120)	Holstein	*Enterococcus faecium* and	15	Milk replacer	
2001	19	0.890 (0.098 to 1.682)	0.000 (−0.900 to 0.900)	Holstein	*Saccharomyces cerevisiae*	15	Milk replacer	

2002	41	0.606 (0.045 to 1.168)	n.d.	Holstein	*Lactobacillus acidophilus*	42	Whole milk	Abdala et al. (2002)
2002	52	−0.014 (−0.557 to 0.529)	n.d.	Holstein		42	Milk replacer	
2003	22	0.404 (−0.443 to 1.251)	0.652 (−0.208 to 1.513)	Holstein	*Lactobacillus plantarum*, *L. bulgaricus*, *L. acidophilus*, *L. mamnsus*, *Bifidobacterium bifidum*, *Streptococcus thermophilus*, *Enterococcus faecium*, *Aspergillus oryza* and *Candida pintolopessi*	60	Whole milk	Gorgulu et al. (2003)
2004	19	3.305 (1.921 to 4.688)	−5.565 (−7.551 to −3.580)	Holstein	*Saccharomyces cerevisiae*, *Lactobacillus acidophilus*, *bifidobacterium bifidum*, *Streptococcus termophilus* and *Aspergillus niger*	180	Whole milk	Isik et al. (2004)
2005	360	0.018 (−0.547 to 0.584)	−5.468 (−6.801 to −4.136)	Holstein	*L. acidophilus*, *L. salivarius*,	53	Milk replacer	Timmerman et al. (2005)
2005	62	0.120 (−0.445 to 0.687)	−5.155 (−6.427 to −3.882)	Holstein	*L. paracasei* spp. *paracasei*,	56		
2005	48	0.409 (−0.209 to 1.028)	−0.105 (−0.364 to 0.152)	Holstein	*L. plantarum*, *L. lactis* and	187		
2005	48	0.279 (−0.336 to 0.894)	−0.510 (−1.016 to −0.004)	Holstein	*Enterococcus faecium*	187		
2005	41	0.046 (−0.211 to 0.304)	0.000 (−0.565 to 0.565)	Holstein		56		
2005	41	0.195 (−0.303 to 0.694)	−0.144 (−0.711 to 0.421)	Holstein	*Lactobacillus* spp.	56		
2006	20	0.615 (−0.281 to 1.512)	0.158 (−0.719 to 1.036)	Holstein	*Bacillus subtillis* and *Bacillus lishniformis*	49	Whole milk (2 l twice per day)	Bakhshi et al. (2006)
2006	20	−0.273 (−1.154 to 0.607)	−0.143 (−1.021 to 0.733)	Holstein	*Bacillus subtillis* and *Bacillus lishniformis*	49	Whole milk (4 l one time per day)	
2007	120	0.544 (0.166 to 0.921)	n.d.	Not specified	*Bifidobacterium bifidum*, *Enterococcus faecium*, *Streptococcus thermophilus*, *Aspergillus oryzae* and *Candida pinotopesti*	90	Whole milk	Mokhber-Dezfouli et al. (2007)
2008	24	0.000 (−0.800 to 0.800)	−0.217 (−1.020 to 0.584)	Holstein	*Lactobacillus casei*, *L. salivarius* and *Pediococcus acidilactici*	35	Milk replacer	Frizzo et al. (2008)
2010	16	5.502 (3.358 to 7.645)	−0.166 (−1.148 to 0.815)	Holstein	*Lactobacillus casei*, *L. salivarius* and *Pediococcus acidilactici*	35	Milk replacer	Frizzo et al. (2010)

[a] SMD standardized mean difference between the probiotic treatment and controls (mean and 95% CI).
n.d. = no data.

Table 2
Meta-analysis comparing probiotics and control on growth rate and feed efficiency of young calves.

Outcomes	Global effect and subgroup analysis restricted	*Trials*[a]	*n*[b]	SMD[c]	95% confidence intervals	*Q*-statistic (P)	I^2	I^2 95% confidence intervals
Body weight gain	Global effect	36	1547	0.228	0.100 to 0.463	<0.0001	0.599	0.522–0.664
	Whole milk	12	418	0.306	−0.079 to 0.691	0.0003	0.681	0.579–0.757
	Milk replacer	24	1129	0.267	0.062 to 0.471	0.0005	0.556	0.449–0.642
	<45 d	12	454	0.243	−0.118 to 0.605	<0.0001	0.699	0.604–0.771
	>45 d	24	1093	0.310	0.099 to 0.521	0.0008	0.543	0.433–0.632
	<20 calves	13	227	0.634	0.038 to 1.231	<0.0001	0.769	0.703–0.820
	>20 calves	23	1320	0.204	0.054 to 0.354	0.0573	0.340	0.176–0.471
	Monostrain	9	353	0.368	0.041 to 0.695	0.0240	0.546	0.380–0.668
	Multistrain	27	1194	0.252	0.032 to 0.472	<0.0001	0.622	0.539–0.690
Feed efficiency	Global effect	14	1117	−0.814	−1.222 to −0.405	<0.0001	0.878	0.857–0.895
	Whole milk	6	125	−0.613	−1.66 to 0.437	<0.0001	0.855	0.800–0.895
	Milk replacer	20	992	−0.886	−1.399 to −0.433	<0.0001	0.886	0.865–0.904
	<45 d	9	273	−0.524	−1.135 to 0.086	<0.0001	0.822	0.765–0.866
	>45 d	17	844	−0.995	−1.545 to −0.445	<0.0001	0.899	0.880–0.916
	<20 calves	9	159	−0.541	−1.186 to 0.104	0.0002	0.724	0.626–0.796
	>20 calves	17	958	−0.936	−1.454 to −0.419	<0.0001	0.908	0.891–0.923
	Monostrain	5	148	−0.554	−1.074 to −0.035	0.0515	0.575	0.390–0.703
	Multistrain	21	969	−0.898	−1.392 to −0.404	<0.0001	0.897	0.879–0.912

[a] Number of comparisons.
[b] Number of calves included in the comparisons.
[c] SMD standardized mean difference between probiotic treatment and controls.

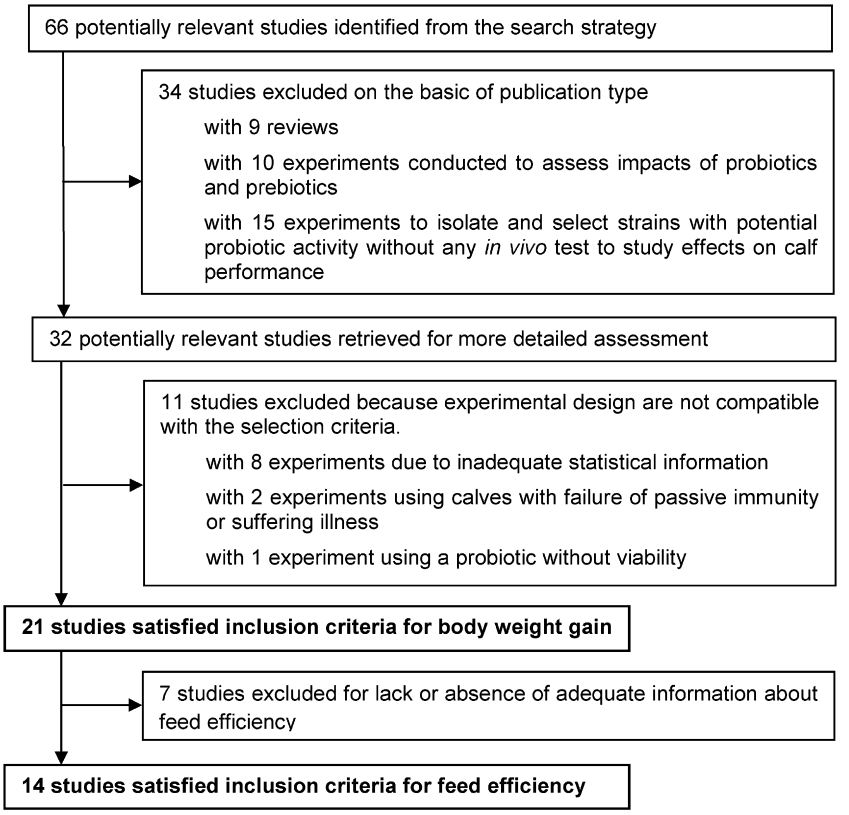

Fig. 1. Study selection flow chart.

Taking into account the duration of the experiments, the subgroup analysis indicated no effect when it was <45 d (SMD = 0.2433, 95% CI −0.1187 to 0.6052), while the beneficial impact remained in those experiments with a duration >45 d.

Subgroup analyses considering the LAB strain used (*i.e.*; *L. plantarum*, *L. salivarius*, *Bifidobacterium* spp., *L. casei*/*paracasei*) did not result in a probiotic effect. Moreover, the probiotic effect remained in those experiments which used *L. acidophilus* or *E. faecium* as the probiotic strain. Finally, the probiotic effect occurred in experiments which used monostrain and multistrain probiotics (Table 2). The subgroup analysis showed that the probiotic effect occurred in experiments which used less or more than 20 calves, but the deviation of the data around the average global estimation was higher in experiments with <20 calves than in those with >20 (Table 2).

Meta-regressions conducted to analyze effects of the number of calves used in the experiments, and their duration, as prediction variables, showed that neither variable was a predictor of effects. The regression coefficients for the number of calves and duration of the experiment were 0.02 (P=0.15) and 0.004 (P=0.54), respectively.

No publication bias occurred for these 36 experiments as confirmed by Begg's test (P=0.14). However, Egger's test was significant (P=0.04). Two studies appeared to have a large influence on the data (Frizzo et al., 2010; Isik et al., 2004). The Sensibility Analysis showed that the beneficial effect of probiotic on BWG remained unchanged when these studies were removed: Frizzo et al. (2010) (SMD = 0.2401; 95% CI 0.0826–0.3976) and Isik et al. (2004) (SMD = 0.2351; 95% CI 0.0790–0.3903).

3.4. Feed efficiency

From the 14 studies that met inclusion criteria we identified 26 experiments (1117 calves) that evaluated effects of probiotics on feed efficiency. The meta-analysis (Table 2) showed that calves fed probiotics had improved feed efficiency in comparison to those without probiotic supplementation (SMD = −0.8141, 95% CI −1.2222 to −0.4059).

Heterogeneity occurred among the 26 experiments (*Q*-statistic: P<0.0001; I^2-statistic: 87.80%, 95% CI 85.78–89.53%), and subgroup analyses revealed that 4 experiments were responsible for the majority of the heterogeneity (Timmerman et al., 2005; Isik et al., 2004; Meyer et al., 2001; Higginbotham and Bath, 1993). When these were removed from the analysis, a homogenous group of 21 experiments (*Q*-statistic: P=0.2878; I^2-statistic: 13.11%, 95% CI 0–27.1%) reveled that the pooled estimate in the feed efficiency was similar (SMD = −0.1845, 95% CI −0.3241 to −0.0449).

A subgroup analysis restricted to type of feed provided to the calves (*i.e.*; whole milk and milk replacer), was conducted. When the experiments which used whole milk were evaluated, probiotics did not impact feed efficiency (SMD = −0.613,

95% CI −1.663 to 0.437; Table 2). However, analyzing the experiments which used milk replacer, a beneficial effect occurred (SMD = −0.8868, 95% CI −1.3999 to −0.4337; Table 2).

Taking into account the duration of the experiments (Table 2), no effect occurred when they were <45 d (SMD = −0.5243, 95% CI −1.1354 to 0.0868) and >60 d (SMD = −0.7757, 95% CI −1.6412 to 0.0898). However, the probiotic effect remained in those studies with a duration >45 d (SMD = −0.9953, 95% IC −1.5452 to −0.4455) and 60 d (SMD = −0.8291, 95% CI −1.3061 to −0.3520).

Considering the LAB strain used in the studies, no effects occurred in those experiments which used *L. plantarum* and those which used *L. acidophilus*. In any other situation, the probiotic effect occurred, even in studies that used monostrain or multistrain probiotics (Table 2).

There was no random global effect when feed efficiency was assessed based on experiments with <20 calves (SMD = −0.5414, 95% CI −1.1869 to 0.1040; Table 2). However, an effect occurred in those which used >20 (SMD = −0.9368, 95% CI −1.4540 to −0.4196).

Meta-regressions conducted to analyze the effect of number of calves used in the experiments, and the duration of the experiment as prediction variables reveled that duration was not a predictor of the study effects. However, the number of calves had an overall significance (P=0.008), but with a low regression coefficient ($R = 0.13$).

A publication bias occurred for these 26 experiments as confirmed by Egger's test (P=0.009) and Begg's test (P=0.002). Three studies appeared to have a large influence on the data (*i.e.*; Meyer et al., 2001; Isik et al., 2004; Timmerman et al., 2005) but, when these were removed from the analysis, the pooled estimate of feed efficiency was similar.

4. Discussion

This quantitative meta-analysis of data from several randomized controlled experiments showed that probiotic supplementation increased BWG and feed efficiency in young calves.

4.1. Probiotic effect

Growth performance of young calves is strongly related to the type of feed which they consume, the rearing system and the intestinal microbiota balance. Probiotics may prevent intestinal microbial imbalances which are common in intensive rearing systems to reduce the incidence of disease. If calves become ill during the first few weeks of life, growth may decrease and result in death or poor productivity, even after they become adults (Ishihara et al., 2001). Young calves are especially susceptible to intestinal infectious diseases during these periods and maintaining or increasing BWG could enhance the resistance to disease (Cruywagen et al., 1996). This increase in both BWG and disease resistance places the young calf in a very favorable situation in which it can continue to gain BW and be better prepared to resist diarrheal pathogens. Results of our meta-analysis show that probiotic administration had a beneficial effect on BWG (228 g/d) and on feed efficiency (814 g less feed consumed/kg of DWG). Different mechanisms of action of probiotics have been described (Fuller, 1989; Blum et al., 1999), which can be summarized as: probiotics compete for nutrients and produce antibacterial compounds (*e.g.*, organic acids, hydrogen peroxide, bacteriocins) in the intestinal lumen allowing them to occupy specific niches of the intestinal mucosa and activate the innate immune system of calves. The involvement of each of these mechanisms is directly related to the type of probiotic strain and feed consumed by the calves. The improvement in utilization of feed and the consequent improvement in BWG is the final consequence of probiotic action.

Results demonstrate that the probiotic effect was more evident during the first few weeks of life and this was especially clear in feed efficiency. Timmerman et al. (2005) report a clear increase in BW gain in 1 week old veal calves supplemented with probiotics but limited beneficial effects during the first 2 weeks of life. Probiotic function may be related to an improvement in feed efficiency, especially in diets containing a high proportion of dry matter as grain and forage (Frizzo et al., 2010), which has positive effect on ruminal development. An improvement in growth during this stage has a large impact on performance in subsequent rearing. This improvement in performance produced by probiotics could help to improve production and economic indices of farms.

4.2. Number of calves

The number of calves is a limiting factor in many experiments and it impacted directly results variability. In this meta-analysis, the probiotic effect on BWG was evident both in experiments with more or less than 20 calves. However, the probiotic effect on feed efficiency was only in experiments which used more than 20 calves.

4.3. Type of inocula

An important point of this meta-analysis is related to utilization of monostrain or multistrain inoculum. Because activity of probiotic microorganisms may vary among calves of the same species, inoculum one often administered as a mixture of strains (Gardiner et al., 2004) since the functionality of a multistrain probiotic inoculum could be more effective and consistent than a monostrain (Timmerman et al., 2005). An advantage of a multistrain inoculum is the possibility of complementary effects of their probiotic properties. This complimentarity of strain roles within a complex ecosystem, such as

the gastrointestinal tract, makes its colonization by multistrain probiotic inoculum more likely than for monostrains (Frizzo et al., 2006). However, in this meta-analysis growth performance was not related to use of monostrain *versus* multistrain inoculum.

4.4. Probiotic strain

Some authors have completed meta-analysis focused on a specific microorganisms (Szajewska et al., 2007), considering that the beneficial effect would be specific to strain. However, there are no commercial probiotics widely distributed worldwide for young calves and, for that reason, it is difficult to find experiments that used the same strain. In contrast, many strains of microorganisms have been used in experiments included in this meta-analysis. Although the sub-analysis identified differences among LAB strains used as probiotics, the results are inconclusive. More studies using similar experimental designs comparing specific microorganisms should be conducted (McFarland et al., 2006).

4.5. Duration of the studies

The probiotic impact on feed efficiency was identified in experiments which were conducted for 45–60 d. The probiotic effect linked to BWG also responded in the first few weeks of life, but there was no beneficial effect before 45 d. However even after 60 d, the effect on BWG may be influenced by probiotic supplementation. This improvement in BWG after 60 d was not accompanied by an improvement in feed efficiency because, in this stage, the calves begin consumption of feed with more fiber.

4.6. Type of liquid diet

Use of milk replacer and feed concentrates during the first few weeks of life may predispose calves to nutritional diarrheas and increase animal stress. In the restricted subgroup analysis we found that probiotic efficacy was feed-related because the positive effect only occurred when calves were fed milk replacer. It is interesting to note that although calves fed whole milk gained more BW (306 g BWG) than those fed milk replacer (267 g BWG), the probiotic effect only occurred in those fed milk replacer. When calves are fed whole milk, health and nutritional problems are less frequent and it is a possible explanation for probiotic inefficacy in our meta-analysis. The beneficial effect of probiotics on growth performance may only be present when their health status is compromised (Timmerman et al., 2005). Use of probiotics would not be justified on farms using whole milk to feed calves, at least relative to growth.

4.7. Source of heterogeneity, publication bias and sensitivity analysis

This meta-analysis included studies conducted in different regions of the world. In this sense, the generalization and validity of the conclusions reached are strong. However, potential biases, such as publication bias and a variety of sources of heterogeneity that have been mentioned by McFarland et al. (2006) may be applicable to our meta-analysis. Restricted subgroup analysis and meta-regressions conducted in our meta-analysis were not able to completely identify sources of heterogeneity.

5. Conclusions

This meta-analysis included a large number of experiments which assessed growth performance of calves and showed that supplementation with LAB is effective in improving BWG and feed efficiency, especially during the first 60 d of rearing in calves fed milk replacer. The probiotic effect was not related to supplementation with monostrain or multistrain inocula. A beneficial effect on feed efficiency was associated with experiments which used >20 calves, while the effect on BWG was identified with <20 calves. Probiotics may be an alternative to use of antibiotics as growth promoters in calves. The need for more research on comparisons among specific probiotic microorganisms is highlighted by our results.

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