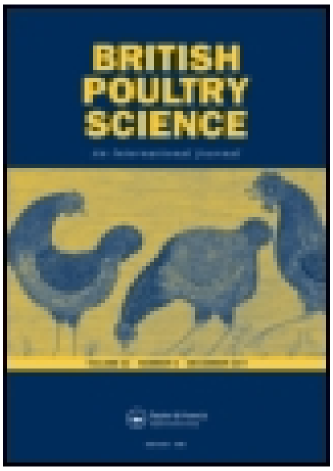

British Poultry Science
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/cbps20

Probiotics and broiler growth performance: a meta-analysis of randomised controlled trials

J.E. Blajman[a], L.S. Frizzo[ab], M.V. Zbrun[ab], D.M. Astesana[a], M.L. Fusari[b], L.P. Soto[ab], M.R. Rosmini[b] & M.L. Signorini[bc]

[a] Laboratory of Food Analysis, Institute of Veterinary Science of the Litoral, National Council of Scientific and Technical Research (ICIVET-CONICET/UNL), Esperanza, Santa Fe, Argentina

[b] Department of Public Health, Faculty of Veterinary Science, National University of the Litoral, Esperanza, Santa Fe, Argentina

[c] National Council of Scientific and Technical Research (CONICET), National Institute of Agricultural Technology (INTA) EEA Rafaela, Santa Fe, Argentina

Accepted author version posted online: 12 Jun 2014.Published online: 22 Aug 2014.

To cite this article: J.E. Blajman, L.S. Frizzo, M.V. Zbrun, D.M. Astesana, M.L. Fusari, L.P. Soto, M.R. Rosmini & M.L. Signorini (2014): Probiotics and broiler growth performance: a meta-analysis of randomised controlled trials, British Poultry Science, DOI: 10.1080/00071668.2014.931930

To link to this article: http://dx.doi.org/10.1080/00071668.2014.931930

British Poultry Science, 2014
http://dx.doi.org/10.1080/00071668.2014.931930

Probiotics and broiler growth performance: a meta-analysis of randomised controlled trials

J.E. BLAJMAN[1], L.S. FRIZZO[1,2], M.V. ZBRUN[1,2], D.M. ASTESANA[1], M.L. FUSARI[2], L.P. SOTO[1,2], M.R. ROSMINI[2] AND M.L. SIGNORINI[2,3]

[1]*Laboratory of Food Analysis, Institute of Veterinary Science of the Litoral, National Council of Scientific and Technical Research (ICIVET-CONICET/UNL), Esperanza, Santa Fe, Argentina,* [2]*Department of Public Health, Faculty of Veterinary Science, National University of the Litoral, Esperanza, Santa Fe, Argentina, and* [3]*National Council of Scientific and Technical Research (CONICET), National Institute of Agricultural Technology (INTA) EEA Rafaela, Santa Fe, Argentina*

Abstract 1. The aim of this meta-analysis was to investigate the effects of probiotics on the growth performance of broilers. PubMed, Scopus and Scholar Google databases were searched in all languages from 1980 to 2012. The studies in the meta-analysis were only selected if they were randomised and controlled experiments using broilers without apparent disease and the results were published in peer-reviewed journals.

2. A total of 48 and 46 studies were included to assess probiotic effects on body weight gain (BWG) and feed efficiency (FE), respectively. Probiotics increased BWG compared to controls (SMD = 0.661, 95% CI 0.499 to 0.822) and improved FE (SMD = – 0.281, 95% CI –0.404 to –0.157) in the pooled standardised mean difference random effect model, considering the source of heterogeneity and publication biases. However, there are evidences of publication bias and heterogeneity, so the results of this meta-analysis should be considered with caution. Applying the Duval and Tweedie's trim-and-fill methods, the adjusted value for BWG was 0.0594 (95% CI –0.122 to 0.242), and the adjusted value for FE did not show any modifications.

3. The meta-analysis showed that application of probiotics *via* water resulted in greater BGW and FE than administration through the feed. The effect was not related to the use of mono-strain or multi-strain probiotics, although it may depend on the strain used. The number of broilers and the duration of the experiments had an impact on the outcomes.

4. Additional studies should be conducted with the aim to identify the covariates which can explain the differences in the estimated effect sizes.

INTRODUCTION

Broilers raised under intensive conditions are exposed to various stressors. Increased stress will manifest in many ways, most commonly in a reduction of overall performance and in a high susceptibility to disease (Rosales, 1994).

A common practice in poultry production has been the addition of antibiotics to feeds used as growth promoters (Bedford, 2000; Joerger, 2003). However, the usefulness of antibiotics has been questioned because of the emergence and spread of antibiotic-resistant bacteria in meat (Patterson and Burkholder, 2003; Huyghebaert *et al.*, 2011). Thus, there is a renewed interest in finding viable alternatives to antibiotics.

Probiotics are live microorganisms which, when administered in adequate amounts, may confer a health benefit on the host (Food and Agriculture Organization (FAO), 2001). Yet no agreement has been reached as to whether probiotics are effective in improving animals' growth

Correspondence to: L.S. Frizzo, National Council of Scientific and Technical Research (CONICET), Department of Public Health, Faculty of Veterinary Science, Universidad Nacional del Litoral, Esperanza, Argentina. E-mail: lfrizzo@fcv.unl.edu.ar
Accepted for publication 24 March 2014.

performance. Many authors reported that the probiotic supplementation repairs the deficiencies in the gut flora and inhibits disturbance of the intestinal microbiota, increasing resistance to infection (Fuller, 1989; Blecha, 2000; Soderholm and Perdue, 2001; Frizzo *et al.*, 2010; Signorini *et al.*, 2012).

A systematic review of the literature constitutes a scientific technique of reviewing the available bibliography about the topic under study, and then using explicit methods in order to identify, select and critically evaluate the studies that are relevant (Faria Filho *et al.*, 2006).

The objective of this meta-analysis has been to assess the effect of probiotic supplementation on body weight gain (BWG) and feed efficiency (FE) of broilers.

MATERIALS AND METHODS

Criteria for study selection

The studies in this meta-analysis were selected only if they were randomised and controlled experiments using broilers without apparent disease, and results were published in peer-reviewed journals between 1980 and 2012. Probiotics may have been administered *via* drinking water or through the feed. All broiler flocks were managed without antibiotics or growth promoters. Studies must have reported BWG and FE with measures of variance. Assorted reviews, duplicate reports, experiments which used non-viable probiotics and a number of studies which evaluated animals with diseases were excluded. The term "study" refers to a scientific article which can involve one or more experiments (each experiment being a controlled one to compare a particular combination of probiotic-treated and control groups of broilers).

Outcomes and definitions

Supplementation with probiotics was analysed as a tool which may improve BWG and FE in broilers. Data concerning body weight and FE correspond to the whole trial. When the study included more than one probiotic group or different doses of the same probiotic, each probiotic group was compared with the control group separately.

Data sources

PubMed, Scopus and Scholar Google databases from 1980 to 2012 were consulted for articles in all languages. Keywords included broiler*, poultry*, weight gain* FE* and feed conversion ratio*. Abstracts were assessed and articles that met the *a priori* inclusion criteria were utilised.

Data extraction

Information on study design, treatments (strains and type of probiotic administration), number and duration of experiments and outcomes, were extracted from each research report. Depending on the outcomes of each study, the frequency and methodology applied were analysed so as to evaluate the quality of the study. However, no scores were used to exclude studies (Lean *et al.*, 2009).

Statistical analysis

Statistical analysis used Comprehensive Meta-Analysis version 2.2 (2011). Due to continuous variables being analysed, results are presented as standardised mean differences (SMD) between the probiotic treatment and controls with 95% confidence intervals (CIs) using a random effects model. In this model the true effect could vary from experiment to experiment and between-experiment variability (true heterogeneity) as well as sampling error are included (Borenstein *et al.*, 2009).

A meta-regression analysis was performed to explore the sources of heterogeneity in the treatment effects. Meta-regression allows assessing the relationship between year of publication, number of broilers included in the experiments, and duration of the studies as covariates, and BWG and FE as outcomes. Additionally, a cumulative meta-analysis was performed to display how the outcomes shift as a function of the year of publication.

A priori subgroup analyses were planned depending on factors that could potentially influence the magnitude of the treatment: (1) Type of probiotic administration (feed *versus* water), (2) Type of inoculum (mono-strain *versus* multi-strain), (3) Lactic acid bacteria (LAB) strain used as mono-strain inocula (with *Lactobacillus* spp.; with *Streptococcus* spp.; with *Enterococcus* spp.; with *Bacillus* spp. and with *Pediococcus* spp.) and (4) Study duration (rearing from 0 to 42 d *versus* >42 d) considering the typical period of broiler rearing.

Heterogeneity among studies was assessed using the DerSimonian and the Laird test (Q-statistic). The degree of heterogeneity was quantified with the inconsistency index (I^2-statistic; Higgins and Thompson, 2002). A 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 on the analysis outcome (Lean *et al.*, 2009). This consists of completing the same analysis (SMD), but dropping one study in each iteration.

The presence of publication bias was investigated using funnel plots. An adjusted rank correlation test using the Egger method (Egger *et al.*,

1997) and the Begg's test (Begg and Mazumdar, 1994) was used to assess publication bias. Bias was considered to be present if at least one of the statistical methods was significant ($P < 0.10$). If there was any evidence of publication bias, from either the statistical tests or the funnel plot, the "trim-and-fill" method (Duval and Tweedie, 2000) was used to estimate the quantity and magnitude of missing studies and resultant unbiased effect size.

RESULTS

Overview of included studies

The literature search yielded 268 scientific papers on probiotics and broilers. Forty-eight of the 268 screened articles met all inclusion criteria to assess the probiotic effect on BWG, while 46 articles were included in the evaluation of the probiotic effect on FE.

Of the studies which assessed the probiotic effect on BWG, only 12 studies were conducted before 2005 and the remaining 36 after 2005. The number of broilers included in the studies was variable: 17 studies included less than 140 animals and 31 studies included more than 140 animals. A total of 28 studies were conducted using multi-strain probiotics, 15 used mono-strain probiotics and the remaining 5 used mono-strain and multi-strain probiotics in the same study. Probiotics were given to broilers either in the drinking water (4) or added to the feed (40) or incorporated in both of them (3). One study added the probiotic not only in water but also in the feed in the same experiment. Studies were conducted for ≤42 d (41) or >42 d (7).

A total of 46 studies were included to assess impacts of probiotics on FE, with 17 studies conducted before 2005 and 29 after 2005. The number of broilers included in each study was variable, with 41 occasions≤600 and 5 occasions>600. Seventeen studies used mono-strain probiotics, whereas 24 were conducted using multi-strain probiotics and the remaining 5 were carried out with both mono-strain and multi-strain probiotics in the same one. In most of the experiments the probiotic was administered by the diet (39), three studies provided the probiotic in the water and three in both types. One study added the probiotic not only in water but also in the feed in the same experiment. Studies were conducted for ≤42 (40) or >42 d (6).

Excluded studies

Of the 268 studies identified at the beginning of the meta-analysis, 182 failed to meet one or more inclusion criteria. Review articles (48), trials involving other species of animals (99) or other additives (19), studies using probiotics for other purposes (3) or experiments conducted to assess impacts of certain diseases (13) were disregarded. A total of 9 (11 for FE) out of the 86 trials that met the inclusion criteria were excluded due to lack of statistical information to conduct a meta-analysis; non-additional information was requested from the authors. Nine experiments which passed initial screening were excluded because broilers were experimentally infected before probiotic supplementation, whereas two studies were rejected because broilers were subjected to modified environmental conditions. Studies that analysed the efficacy of prebiotics (3), symbiotics (6), non-viable or transformed LAB (3) or yeasts (6) were also excluded from the meta-analysis (Figure 1).

Body weight gain

Of the 48 studies that met the inclusion criteria, 89 experiments (22 738 broilers) that combined broilers fed with probiotics and control groups were identified. In the pooled estimate, probiotics increased BWG compared to controls (SMD = 0.661, 95% CI 0.499 to 0.822) in the pooled SMD random effect model. Significant heterogeneity was observed across the 89 experiments (Q-statistic: $P < 0.0001$; I^2-statistic = 96.99%, 95% CI 96.82% to 97.14%, Figure 2).

Year of publication and number of broilers included in the experiments were associated with the BWG ($P = 0.00009$ and $P < 0.00001$, respectively) in the meta-regression analysis. However, these covariates explained only 0.51% and 5.39%, respectively, of the heterogeneity. Duration of experiments were not associated with BWG ($P = 0.237$) in the meta-regression (Table).

Thirty-four experiments conducted before 2005 were identified, and probiotics increased BWG (SMD = 0.429, 95% CI 0.159 to 0.698; Q-statistic: $P < 0.0001$; I^2-statistic = 97.89%). The 55 experiments conducted after 2005 found a greater beneficial effect in BWG (SMD = 0.799, 95% CI 0.604 to 0.933; Q-statistic: $P < 0.0001$; I^2-statistic = 95.60%).

The average BWG of broilers treated with probiotics and control (broilers without probiotic supplementation) were 2079 g and 1995 g, respectively, with an average standard deviation of 174.25 g. The minimum number of broilers required in an experiment was estimated, considering a 95% CI and a power of 80%. This indicated that experiments should include at least 140 broilers (70 in each group) to identify statistical differences between groups. Experiments which used <140 broilers showed higher BWG than experiments with >140 broilers ($P = 0.013$). The meta-analysis showed that animals fed with probiotics

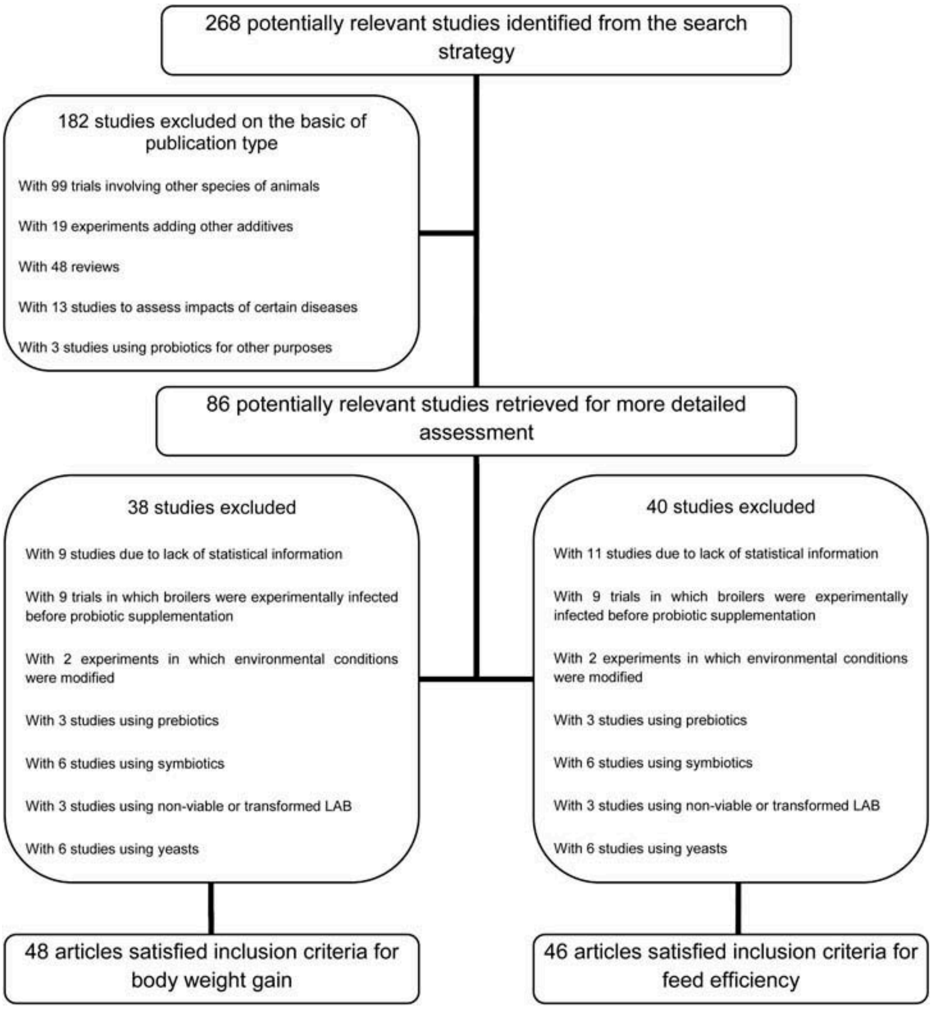

Figure 1. *Study selection flow chart.*

enhanced BWG in experiments with >140 broilers (n = 28; SMD = 0.564, 95% CI 0.387 to 0.741; Q-statistic: $P < 0.0001$; I^2-statistic = 97.38%), and also the probiotic effect was observed in experiments which used less than 140 broilers (n = 61; SMD = 1.211, 95% CI 0.730 to 1.691; Q-statistic: $P < 0.0001$; I^2-statistic = 95.73%, Figure 3).

Probiotic supplementation improved BWG in experiments which used mono-strain (n = 35; SMD = 0.513, 95% CI 0.276 to 0.750; Q-statistic: $P < 0.0001$; I^2-statistic = 97.16%) and multi-strain probiotics (n = 51; SMD = 0.787, 95% CI 0.553 to 1.021; Q-statistic: $P < 0.0001$; I^2-statistic = 96.54%) (P = 0.217). Among the groups of mono-strain inocula, the probiotic effect remained in those experiments which used *Pediococcus* spp. (n = 5; SMD = 2.051, 95% CI 1.187 to 2.915) and *Bacillus* spp. (n = 25; SMD = 0.421, 95% CI 0.156 to 0.686) as the probiotic strain. The effect of probiotic supplementation on BWG remained unchanged when *Lactobacillus* spp. (n = 3; SMD = 0.080, 95% CI −0.173 to 0.334) or *Enterococcus* spp. (n = 1; SMD = 0.126, 95% CI −0.343 to 0.595) was added. With the addition of S*treptococcus* spp., broilers lost weight (n = 1; SMD = −0.746, 95% CI −0.911 to −0.580). However, since the number of experiments that used a certain mono-strain inocula was relatively small, with the exception of *Bacillus* spp., the effects must be interpreted with caution (Figure 3).

Taking into account the duration of the experiments, the subgroup analysis indicated similar positive effects when they were ≤42 d (n = 73; SMD = 0.692, 95% CI 0.513 to 0.870; Q-statistic: $P < 0.0001$; I^2-statistic = 96.92%) or >42 d (n = 16; SMD = 0.525, 95% CI 0.127 to 0.923; Q-statistic: $P < 0.0001$; I^2-statistic = 97.43%) (Figure 3) (P = 0.455).

Finally, considering the method of administration, the beneficial impact remained in those experiments in which probiotics were included in the drinking water (n = 10) or added to the feed (n = 78). Even so, application *via* water resulted in a greater BGW improvement (SMD = 1.369, 95% CI 0.732 to 2.006; Q-statistic: $P < 0.0001$; I^2-statistic = 96.65%) than administration through the feed (SMD = 0.617, 95% CI 0.445 to 0.788; Q-statistic: $P < 0.0001$; I^2-statistic = 97.09%) ($P < 0.001$). BWG was found to be lower in broilers receiving the probiotic both in water and in

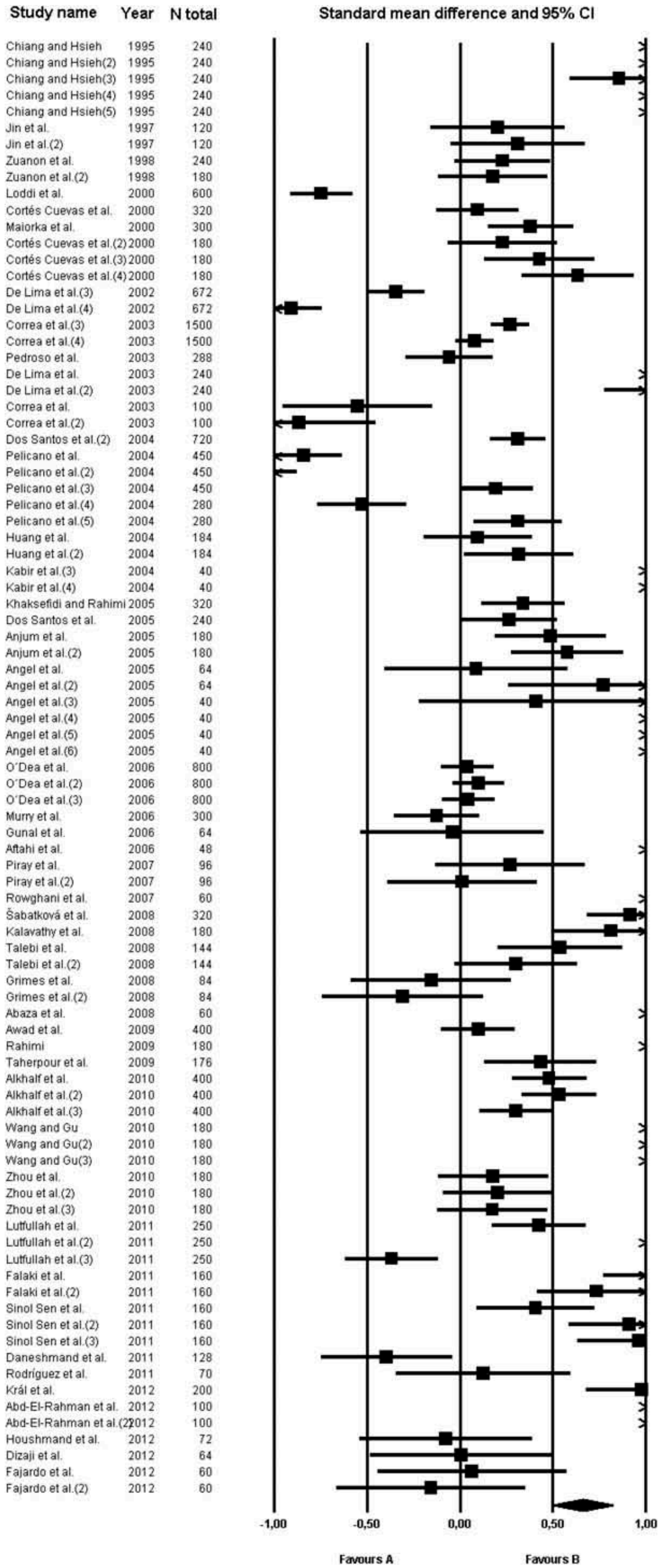

Figure 2. *Forest plot of 89 randomised, controlled trials to study the effect of supplementation with probiotics on body weight gain (BWG) in broilers.*

Table. *Summary of random weighted meta-regression analysis for independent variables that influenced the effects between probiotic supplementation versus no probiotic supplementation in broilers (standardised mean differences, SMD)*

Co-variable		BWG	FE
Year of publication	Intercept	−26.421	76.833
	P	0.00011	<0.00001
	Slope	0.013	−0.038
	P	0.00009	<0.00001
Number of broilers included in the experiments	Intercept	0.481	−0.147
	P	<0.00001	<0.00001
	Slope	−0.00039	0.00001
	P	<0.00001	0.6943
Duration of the experiments	Intercept	0.190	−0.1368
	P	0.0067	0.0502
	Slope	0.0019	−0.0001
	P	0.2372	0.9513

BWG: Body weight gain. FE: Feed efficiency.

feed (SMD = −0.157, 95%Cl −0.288 to 0.174, Figure 3).

A significant publication bias was observed for these 89 experiments, as confirmed by Begg's test ($P < 0.0001$) and Egger's test ($P < 0.0001$). Despite subgroup analysis, in no case, was a reduction of heterogeneity achieved. In this context, methods to assess the publication bias are known to be poor due to the unexplainable heterogeneity. Applying the Duval and Tweedie's trim-and-fill methods identified 29 studies trimmed and the adjusted value for BWG was 0.0594 (95% CI −0.122 to 0.242, Figure 4).

Feed efficiency

From the 46 studies that met inclusion criteria we singled out 90 experiments (25 910 broilers) that evaluated effects of probiotics on FE. The meta-analysis showed that broilers fed with probiotics had improved FE in comparison with those without probiotic supplementation (SMD = − 0.281, 95% CI −0.405 to −0.157) in the pooled SMD random effect model. Heterogeneity occurred among the 90 experiments (*Q*-statistic: $P < 0.0001$; I^2-statistic: 95.67%, 95% CI 95.39% to 95.92%, Figure 5).

Year of publication was significantly associated with FE ($P < 0.000001$) and explained only 7.3% of the heterogeneity (Table). Additionally, the cumulative forest plot sorted

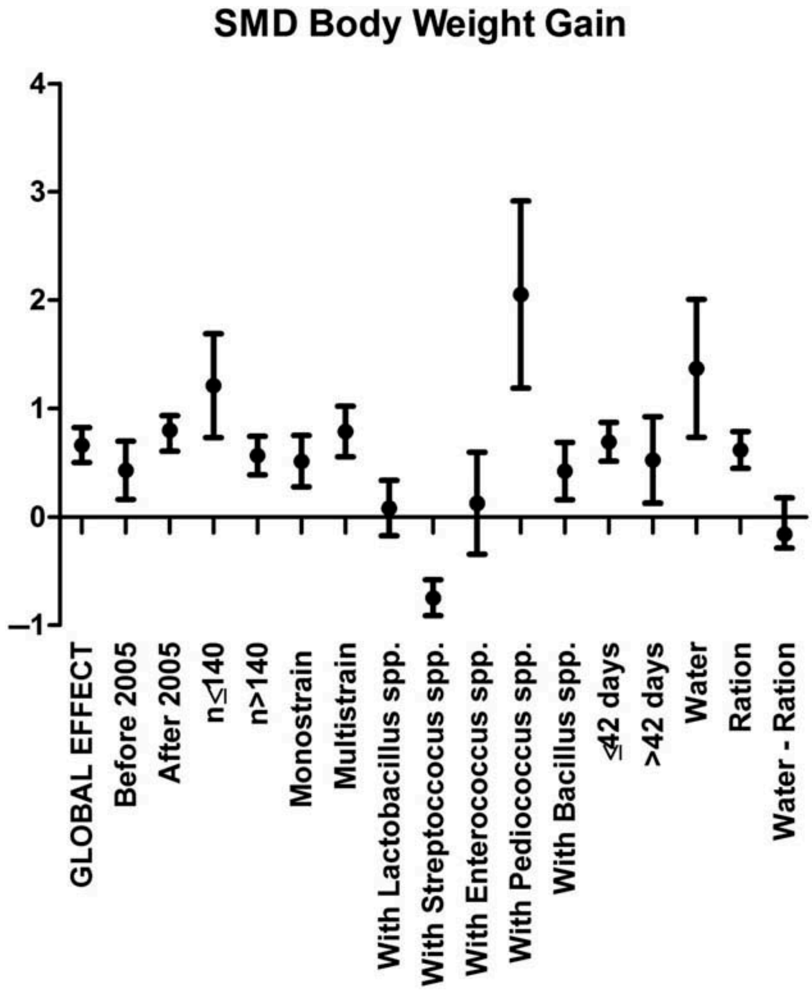

Figure 3. *Subgroup analysis comparing the effect of probiotic supplementation on body weight gain (BWG) of broilers.*

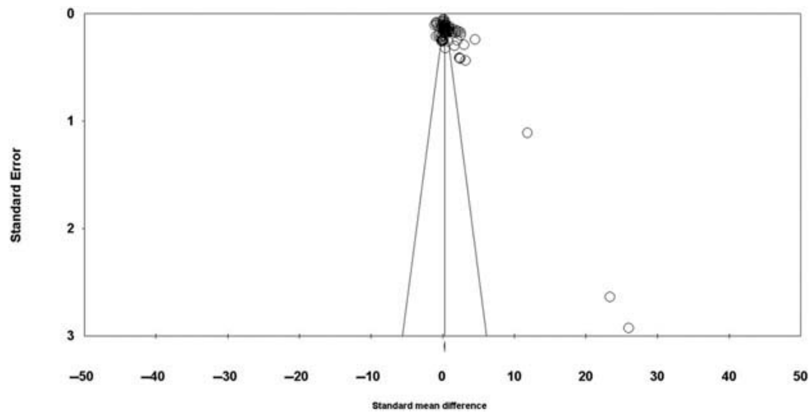

Figure 4. *Funnel plot obtained with the Duval and Tweedie's "Trim-and-Fill" linear random effect model for body weight gain (BWG) as an outcome.*

chronologically showed a clear evidence of relationship between year of publication and FE. Considering only the studies published before 2005, the FE SMD was not significant. A subgroup analysis restricted to year of publication was conducted. When the experiments were carried out before 2005, probiotics did not impact FE (n = 35; SMD = 0.088, 95% CI –0.097 to 0.272; Q-statistic: $P < 0.0001$; I^2-statistic = 96.80%). However, analysing the experiments performed after 2005, a beneficial effect was observed (n = 55; SMD = – 0.531, 95% CI –0.683 to –0.378; Q-statistic: $P < 0.0001$; I^2-statistic = 92.08%, Figure 6). Studies conducted after 2005 showed a higher beneficial effect in the probiotic-treated broilers ($P < 0.001$).

Number of broilers included in the experiments was not associated with the FE (P = 0.694) in the meta-regression analysis (Table). The average FE of broilers treated with probiotics and control (broilers without probiotic supplementation) were 1.901 and 1.950, respectively, with an average standard deviation of 0.207. Using these statistics, the minimum number of broilers required in an experiment was estimated, considering a 95% CI and a power of 80%. Experiments should include at least 600 broilers (300 in each group) to identify statistical differences between groups. Considering the number of animals included in the studies, a probiotic effect was observed when they were >600 (n = 13; SMD = –0.05, 95% CI –0.086 to –0.014; Q-statistic: $P < 0.0001$; I^2-statistic = 97.68%). In the same way, the probiotic effect remained in those studies with ≤600 broilers (n = 77; SMD = – 0.226, 95% CI –0.260 to –0.191; Q-statistic: $P < 0.0001$; I^2-statistic = 94.89%, Figure 6). However, experiments which include <600 broilers showed a higher beneficial effect on the FE (P = 0.007).

The probiotic effect occurred in studies that used both mono-strain (n = 39; SMD = – 0.229, 95% CI –0.421 to –0.037; Q-statistic: $P < 0.0001$; I^2-statistic = 96.94%) and multi-strain probiotics (n = 51; SMD = – 0.321, 95% CI –0.483 to –0.159; Q-statistic: $P < 0.0001$; I^2-statistic = 93.84%) (P = 0.473). In view of the LAB strain used, probiotic treatment decreased FE in those experiments which added *Streptococcus* spp. (n = 1; SMD = 0.171, 95% CI 0.011 to 0.332) and those which used *Enterococcus* spp. (n = 4; SMD = 0.068, 95% CI 0.003 to 0.134). When *Lactobacillus* (n = 3; SMD = – 0.102, 95% CI –0.356 to 0.151) spp. or *Pediococcus* spp. (n = 5; SMD = – 0.215, 95% CI –0.691 to 0.262) were included, no effects were reported on FE. In most situations, a beneficial impact appeared in those which used *Bacillus* spp. (n = 26; SMD = – 0.311, 95% CI –0.596 to –0.026). Since the number of studies that used a certain mono-strain inoculum was relatively small, with the exception of *Bacillus* spp., the effects must be interpreted with extreme care (Figure 6).

Studies that used feed supplemented with probiotics found beneficial effects on FE (n = 81; SMD = – 0.263, 95% CI –0.395 to –0.131; Q-statistic: $P < 0.0001$; I^2-statistic = 95.85%). Studies that used drinking water application as a route of probiotic administration also showed beneficial effects on FE (n = 8; SMD = – 0.454, 95% CI –0.882 to –0.026; Q-statistic: $P < 0.0001$; I^2-statistic = 93.88%, Figure 6) (P = 0.286).

Finally, duration of the experiments was not associated with FE (P = 0.951) in the meta-regression analysis (Table). Experiments that evaluated probiotic administration during ≤42 (n = 75) or >42 d (n = 15) were identified; a positive effect was observed only across the first subgroup analysis (SMD = –0.256, 95% CI –0.370 to –0.142; Q-statistic: $P < 0.0001$; I^2-statistic = 93.87% and SMD = –0.387, 95% CI –0.905 to 0.130; Q-statistic: $P < 0.0001$; I^2-statistic = 98.31%, respectively) (Figure 6). There were no differences between these groups (P = 0.628).

Publication bias occurred for these 90 experiments as confirmed by Egger's test (P = 0.04836)

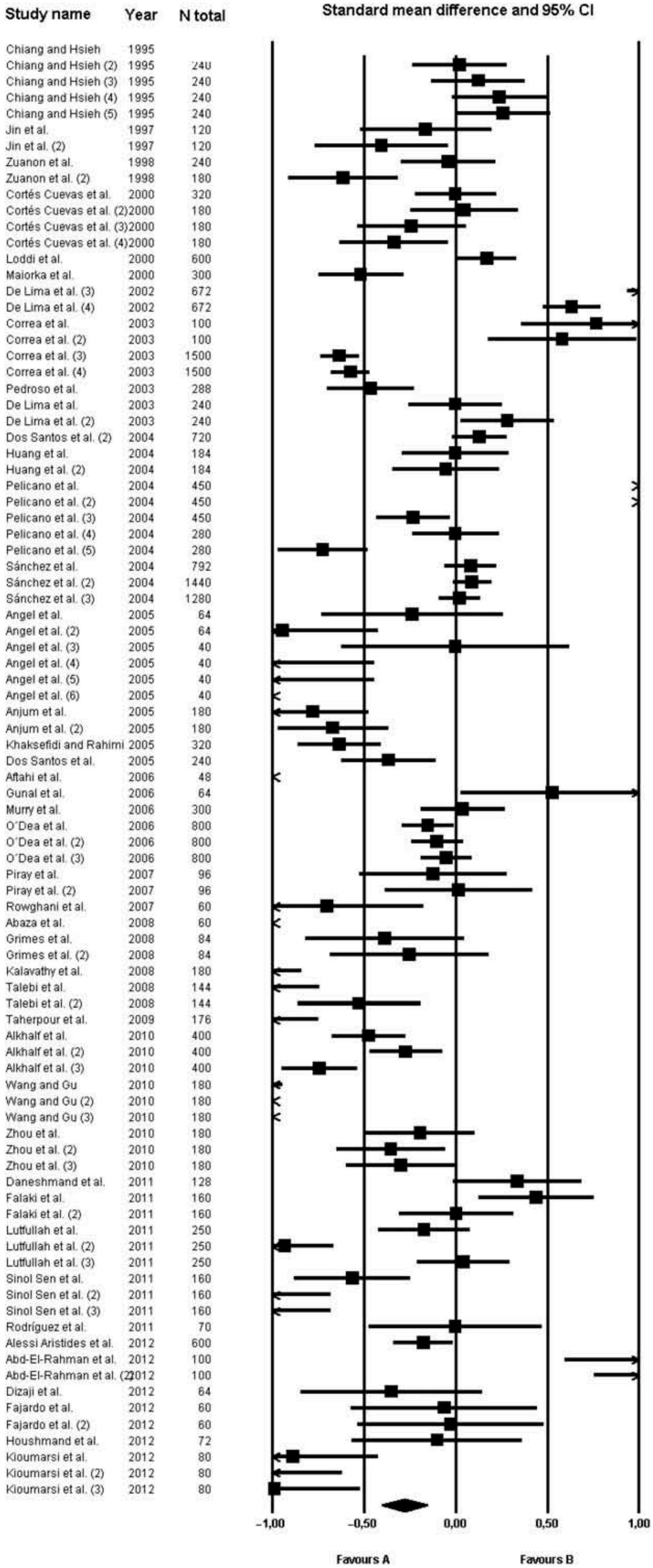

Figure 5. *Forest plot of 90 randomised, controlled trials to study the effect of supplementation with probiotics on FE in broilers.*

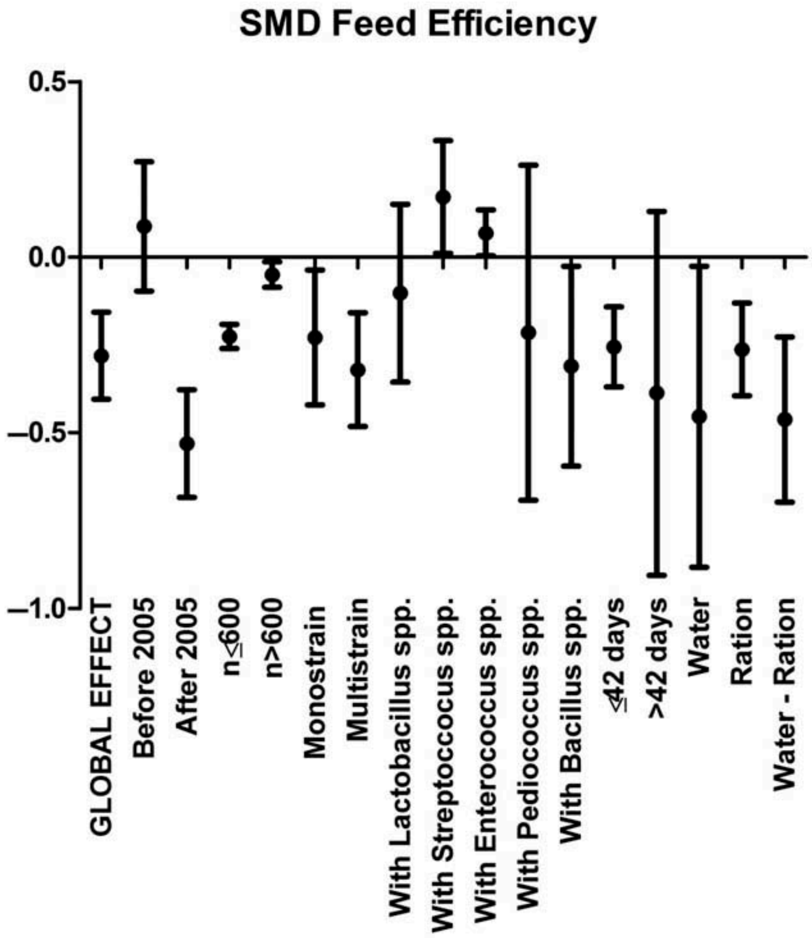

Figure 6. *Subgroup analysis comparing the effect of probiotic supplementation on FE of broilers.*

and Begg's test (P = 0.00603). Applying the Duval and Tweedie's trim-and-fill methods, none of the studies trimmed were identified and thus the adjusted value for FE did not show any modifications (Figure 7). As has been observed in the case of BWG, any subgroup analysis showed a reduction in the heterogeneity achieved. In this context, methods to assess the publication bias are known to be poor due to the unexplainable heterogeneity.

DISCUSSION

Probiotic effect

This quantitative meta-analysis of data from several randomised controlled experiments reveal that compared with the negative control, inclusion of probiotics may improve BWG and FE in broilers.

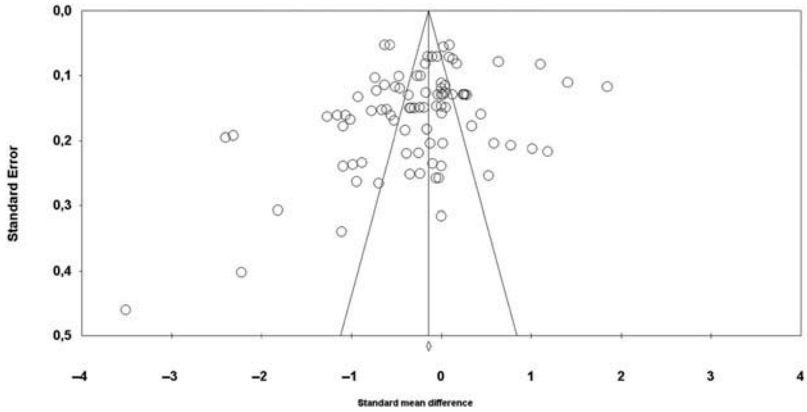

Figure 7. *Funnel plot obtained with the Duval and Tweedie's "Trim-and-Fill" linear random effect model for FE as an outcome.*

When there is a balance between beneficial and non-beneficial intestinal bacteria, broilers perform to their maximum efficiency. However, there are many factors such as feed, environment and stress that could compromise the microbiota (Trachoo and Boudreaux, 2006).

Results of our meta-analysis show that probiotic supplementation had a beneficial effect on BWG (661 g) and on FE (281 g less feed consumed/kg of weight gain). However, the one method of adjustment for bias considerably reduced the effect on BWG. Nevertheless, the effect on FE remained unchanged. Therefore, results should be interpreted with caution.

Different mechanisms of action of probiotics have been proposed: competition for nutrients and production of antibacterial compounds (e.g., organic acids, hydrogen peroxide, bacteriocins), interference with the ability of pathogens to colonise and infect the mucosa and modulation of local and systemic immune responses (Fuller, 1989; Gibson and Fuller, 2000; Rolfe, 2000; Frizzo *et al.*, 2011). Enhanced performance could also be ascribed to the presence of amylase, protease and lipase in probiotic bacteria, which would enhance the catalytic activities of the endogenous enzymes to hydrolyse more energy from feed nutrients (Rahman *et al.*, 2009).

Year of publication

The results of this meta-analysis show a greater benefit in BWG and FE due to probiotic supplementation in studies conducted after 2005. Moreover, adding probiotics has detrimental effects on FE before 2005, but the stratified analysis indicated a beneficial impact after 2005. There are at present more specific tests to characterise and select proper strains. More recent *in vitro* and *in vivo* assays have enabled suitable probiotic strains to be identified. Also, technological aspects have been improved thus allowing for probiotics to be manufactured under industrial conditions, to survive and retain their functionality during storage, and also in the food products into which they are finally formulated (Saarela *et al.*, 2000).

Number of broilers

Probiotic inclusion has significant effect on performance only in experiments which used ≤140 and ≤600 broilers to BWG and FE experiments, respectively. The tendency for probiotic treatment to be less effective could be related to the difficulty of exerting control over variables (probiotic access, doses level, farm hygiene) that is emphasised when the number of broilers increases.

Type of inocula

Another determinant of probiotic efficacy may be the utilisation of mono-strain *versus* multi-strain inocula. Multi-strain probiotics have more chances of successfully colonising the gut tract (Timmerman *et al.*, 2004). Furthermore, a combination of several strains with different mechanisms of action and a greater variety of antimicrobial capacities could perhaps amplify and promote the protective range of bio-therapeutic preparations (Filho-Lima *et al.*, 2000). However, broilers involved in this meta-analysis that received mono-strain or multi-strain probiotic performed similarly.

Probiotic strain

FE did not change due to *Lactobacillus* spp. and *Pediococcus* spp. and decreased with *Streptococcus* spp. and *Enterococcus* spp. Similarly, there was no difference in BWG for broilers supplemented with *Lactobacillus* spp. or *Enterococcus* spp., while broilers treated with *Streptococcus* spp. showed a lower value of BWG. However, the addition of *Pediococcus* spp. and *Bacillus* spp. brought about a positive effect. *Bacillus* spp. may be a good candidate for probiotic use as it has the ability to form spores which are more resistant to heat and facilitates the pelleting process used in the mass production of probiotic broiler feeds (Zhou *et al.*, 2010). With respect to *Pediococcus* spp., it has the potential to exert antagonism against enteric pathogens, primarily through the production of lactic acid and secretion of bacteriocins known as pediocins (Daeschel and Klaenhammer, 1985). Although the stratified analysis identified differences among bacteria strains used as probiotics, the number of studies that used a certain strain was relatively small, with the exception of *Bacillus* spp., and caution should be taken when interpreting the findings. To reach conclusive results, we recommend further research in comparing specific microorganisms.

Duration of studies

In this meta-analysis, the duration of the experiment was not a significant predictor of BWG. However, FE was enhanced only in ≤42 d experiments. It may be envisaged that probiotics exert a better effect during early life, when a stable gut microflora has not yet been established, and therefore, broilers are more vulnerable to environmental pathogens (Gaggìa *et al.*, 2010). Initial colonisation is very relevant to the host because the bacteria can modulate expression of genes in epithelial cells, thus creating a favourable habitat for themselves (Hooper *et al.*, 2001). Therefore, establishment of desirable microorganisms in newly hatched broilers is relevant to the final composition of the permanent flora in full-grown broilers (Timmerman *et al.*, 2006).

Methods administration

Differences in the method of administration of probiotic may be a factor affecting growth performance of variables. Although probiotics have enhanced BWG and FE both *via* the feed and the drinking water, there was a trend towards greater benefits with probiotic administration *via* the drinking water. Eckert *et al.* (2010) determined that post-pelleting feed application may not be as effective as drinking water application as a route of probiotic supplementation. Drinking water appears to be better than the more conventional in-feed supplementation method due to the fact that the LAB are destroyed partly or totally by the current pelleting process (Ghadban, 2002).

Source of heterogeneity and publication biases

This meta-analysis included a large number of experiments, which could allow us to conclude that the use of probiotics improves the growth performance of broilers. Probiotic administration in drinking water increased BWG and FE in comparison with supplemented fed diets. Broilers that received the probiotic for ≤42 d and in groups with ≤140 or ≤600 animals showed higher values of BWG and FE, respectively. The findings of this meta-analysis suggest that the effect of probiotics is not related to the use of mono-strain or multi-strain probiotic, although the aforesaid effect may depend on the strain used. In spite of the previous statement, we are of the belief that further studies are crucial in order to identify potential differences between the probiotics and standardise future trial designs.

However, several publication biases and different sources of heterogeneity are to be considered in this meta-analysis. Restricted subgroup analysis was not enough to completely identify the causes of heterogeneity. Funnel plot asymmetry and regression methods could help to detect small study effects which may be due to publication bias. However, publication bias is only one reason why a funnel plot may appear asymmetric; there are many others covariates that could explain this behaviour (Peters *et al.*, 2010). The effect of factors that are not always described such as viability, storage conditions and safety of the probiotics used, administration level, frequency of application, overall diet, farm hygiene and broiler health status need to be thoroughly assessed because they may explain the differences between the studies reviewed. For those reasons, it is important to emphasise the presence of variation that could not be explained (residual heterogeneity) by the covariates included in this meta-analysis and that these results should be considered with caution.

ACKNOWLEDGMENTS

Marcelo L. Signorini, Laureano S. Frizzo and Lorena P. Soto are Research Career Members from Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET, Argentina). Jesica Blajman, María Virginia Zbrun and Diego Astesana are doctoral fellows from Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET, Argentina).

REFERENCES

Bedford, M. (2000) Removal of antibiotic growth promoters from poultry diets: implications and strategies to minimise subsequent problems. *World's Poultry Science Journal*, **56**: 347–365. doi:10.1079/WPS20000024

Begg, C.B. & Mazumdar, M. (1994) Operating characteristics of a rank correlation test for publication bias. *Biometrics*, **50**: 1088–1101. doi:10.2307/2533446

Blecha, F. (2000) Immune system response to stress, in: Mench, M. & Moberg, G.O. (Eds), *Biology of Animal Stress*, pp. 111–122 (*Oxon, CAB International*).

Borenstein, M., Hedges, L.V., Higgins, J.P.T. & Rothstein, H.R. (2009) *Introduction to Meta-Analysis.* (The Atrium, Southern Gate, Chichester, West Sussex, United Kingdom, John Wiley & Sons, Ltd).

Daeschel, M.A. & Klaenhammer, T.R. (1985) Association of a 13,6 megadalton plasmid in *Pediococcus pentosaceus* with bacteriocin activity. *Applied and Environmental Microbioogy*, **50**: 1538–1541.

Duval, S. & Tweedie, R. (2000) Trim and fill: a simple funnel-plot-based method of testing and adjusting for publication bias in meta-analysis. *Biometrics*, **56**: 455–463. doi:10.1111/j.0006-341X.2000.00455.x

Eckert, N.H., Lee, J.T., Hyatt, D., Stevens, S.M., Anderson, S., Anderson, P.N., Beltran, R., Schatzmayr, G., Mohnl, M. & Caldwell, D.J. (2010) Influence of probiotic administration by feed or water on growth parameters of broilers reared on medicated and nonmedicated diets. *The Journal of Applied Poultry Research*, **19**: 59–67. doi:10.3382/japr.2009-00084

Egger, M., Davey Smith, G., Schneider, M. & Minder, C. (1997) Bias in meta-analysis detected by a simple, graphical test. *BMJ (Clinical Research Ed.)*, **315**: 629–634. doi:10.1136/bmj.315.7109.629

Faria Filho, D.E., Torres, K.A.A., Faria, D.E., Campos, D.M.B. & Rosa, P.S. (2006) Probiotics for broiler chickens in Brazil: systematic review and meta-analysis. *Brazilian Journal of Poultry Science*, **8**: 89–98.

Filho-Lima, J.V.M., Vieira, E.C. & Nicoli, J.R. (2000) Antagonistic effect of *Lactobacillus acidophilus, Saccharomyces boulardii* and *Escherichia coli* combinations against experimental infections with *Shigella flexneri* and *Salmonella enteritidis* subsp. *typhimurium* in gnotobiotic mice. *Typhimurium. Journal of Applied Microbiology*, **88**: 365–370. doi:10.1046/j.1365-2672.2000.00973.x

Food and Agriculture Organization (FAO). (2001) Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria: report of a joint FAO/WHO expert consultation on evaluation of health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria, (Geneva, Food and Agriculture Organization). Available: http://www.who.int/foodsafety/publications/fs_management/en/probiotics.pdf. Accessed 4 Apr 2013.

Frizzo, L.S., Soto, L.P., Zbrun, M.V., Bertozzi, E., Sequeira, G., Rodríguez Armesto, R. & Rosmini, M.R. (2010) Lactic acid bacteria to improve growth performance in young calves fed milk replacer and spray-dried whey powder. *Animal Feed Science and Technology*, **157**: 159–167. doi:10.1016/j.anifeedsci.2010.03.005

Frizzo, L.S., Zbrun, M.V., Soto, L.P. & Signorini, M.L. (2011) Effects of probiotics on growth performance in young calves: a meta-analysis of randomized controlled trials. *Animal Feed Science and Technology*, **169**: 147–156. doi:10.1016/j.anifeedsci.2011.06.009

Fuller, R. (1989) Probiotics in man and animals. *Journal of Applied Bacteriology*, **66**: 365–378. doi:10.1111/j.1365-2672.1989.tb05105.x

Gaggìa, F., Mattarelli, P. & Biavati, P. (2010) Probiotics and prebiotics in animal feeding for safe food production. *International Journal of Food Microbiology*, **141**: SS15–SS28. doi:10.1016/j.ijfoodmicro.2010.02.031

Ghadban, G.S. (2002) Probiotics in broiler production – a review. *Archives Geflu¨Gelk*, **66**(2): 49–58.

Gibson, G.R. & Fuller, R. (2000) Aspects of in vitro and in vivo research approaches directed toward identifying probiotics and prebiotics for human use. *Journal of Nutrition*, **130**: 391–395.

Higgins, J.P.T. & Thompson, S.G. (2002) Quantifying heterogeneity in a meta-analysis. *Statistics in Medicine*, **21**: 1539–1558. doi:10.1002/sim.1186

Hooper, L.V., Wong, M.H., Thelin, A., Hansson, L., Falk, P.G. & Gordon, J.I. (2001) Molecular analysis of commensal host microbial relationships in the intestine. *Science*, **291**: 881–884. doi:10.1126/science.291.5505.881

Huyghebaert, G., Ducatelle, R. & Van Immerseel, F. (2011) An update on alternatives to antimicrobial growth promoters for broilers. *The Veterinary Journal*, **187**: 182–188. doi:10.1016/j.tvjl.2010.03.003

Joerger, R.D. (2003) Alternatives to antibiotics: bacteriocins, antimicrobial peptides and bacteriophages. *Poultry Science*, **82**: 640–647. doi:10.1093/ps/82.4.640

Lean, I.J., Rabiee, A.R., Duffield, T.F. & Dohoo, I.R. (2009) Invited review: use of meta-analysis in animal health and reproduction: methods and applications. *Journal of Dairy Science*, **92**: 3545–3565. doi:10.3168/jds.2009-2140

Patterson, J.A. & Burkholder, K.M. (2003) Application of prebiotics and probiotics in poultry production. *Poultry Science*, **82**: 627–631. doi:10.1093/ps/82.4.627

Peters, J.L., Sutton, A.J., Jones, D.R., Abrams, K.R., Rushton, L. & Moreno, S.G. (2010) Assessing publication bias in meta-analyses in the presence of between-study heterogeneity. *Journal of the Royal Statistical Society: Series A (Statistics in Society)*, **173**: 575–591. doi:10.1111/j.1467-985X.2009.00629.x

Rahman, A.U., Sarzamin, K., Daulat, K., Hussain, M., Ahmed, S., Sohail, S.M., Ahmed, I., Haq, I.U. & Shah, Z. (2009) Use of probiotics in broiler feed at starter phase. *Sarhad Journal of Agriculture*, **25***(3)*: 469–474.

Rolfe, R.D. (2000) The role of probiotic cultures in the control of gastrointestinal health. *Journal of Nutrition*, **130**: 396–402.

Rosales, G. (1994) Managing stress in broiler breeders: a review. *The Journal of Applied Poultry Research*, **3**: 199–207. doi:10.1093/japr/3.2.199

Saarela, M., Mogensen, G., Fondén, R., Mättö, J. & Mattila-Sandholm, T. (2000) Probiotic bacteria: safety, functional and technological properties. *Journal of Biotechnology*, **84**: 197–215. doi:10.1016/S0168-1656(00)00375-8

Signorini, M.L., Soto, L.P., Zbrun, M.V., Sequeira, G.J., Rosmini, M.R. & Frizzo, L.S. (2012) Impact of probiotic administration on the health and fecal microbiota of young calves: a meta-analysis of randomized controlled trials of lactic acid bacteria. *Research in Veterinary Science*, **93**: 250–258. doi:10.1016/j.rvsc.2011.05.001

Soderholm, J.D. & Perdue, M.H. (2001) Stress and the gastrointestinal tract II. Stress and intestinal barrier function. *American Journal of Physiology – Gastrointestinal and Liver Physiology*, **28**: G7–G13.

Timmerman, H.M., Koning, C.J.M., Mulder, L., Rombouts, F.M. & Beynen, A.C. (2004) Monostrain, multistrain and multispecies probiotics. A comparison of functionality and efficacy. *International Journal of Food Microbiology*, **96**: 219–233. doi:10.1016/j.ijfoodmicro.2004.05.012

Timmerman, H.N., Veldman, A., Vanden Elsen, E., Rombouts, F.M. & Beynen, A.C. (2006) Mortality and growth performance of broilers given drinking water supplemented with chicken-specific probiotics. *Poultry Science*, **85**: 1383–1388. doi:10.1093/ps/85.8.1383

Trachoo, N. & Boudreaux, C. (2006) Therapeutic properties of probiotic bacteria. *Journal of Biological Sciences*, **6**: 202–208. doi:10.3923/jbs.2006.202.208

Zhou, X., Wang, Y., Gu, Q. & Li, W. (2010) Effect of dietary probiotic, Bacillus coagulans, on growth performance, chemical composition, and meat quality of Guangxi Yellow chicken. *Poultry Science*, **89**: 588–593. doi:10.3382/ps.2009-00319.