Nutritional Impact of Dietary Plasma Proteins in Animals Undergoing Experimental Challenge and Implications for Patients with Inflammatory Bowel Disorders: A Meta-analysis[1,2]

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ABSTRACT

Studies administering plasma protein isolates (PPIs) to experimentally challenged animals have reported improvements in growth, food intake, and overall condition when compared with animals fed control diets, due in part to improvements in gut barrier function, normalization of cytokine signals, and support of enteric immune function. These and early clinical studies suggest that nutritional therapy with PPIs may similarly assist in restoring homeostasis to gut barrier function in humans experiencing mild or more acute enteropathic symptomatology such as irritable bowel syndrome and inflammatory bowel disease. This meta-analysis evaluated the ability of PPIs to promote weight gain and food intake in weanling animals, primarily piglets, after oral challenge with various enteric pathogens or bacterial toxins. MEDLINE, EMBASE, and PubMed were searched from 1980 through August 2012 for specified terms and keywords. Twenty-nine articles retrieved through this process were evaluated; 11 studies including 13 experiments were selected for inclusion in the analysis. The meta-analysis included descriptive analyses and methods for combining P values for the primary endpoint, average daily growth (ADG) at week 1, and secondary endpoints including ADG, average daily feed intake (ADFI), and gain to feed ratio (G:F) at weeks 1 and 2 and at the end of study. Primary and secondary endpoint analyses of growth (ADG, ADFI, and G:F) were significant ($P < 0.01$). The proinflammatory cytokines interleukin (IL) 1β, IL-6, and tumor necrosis factor α were significantly lower in animals fed dietary PPIs. Additional research in patients experiencing symptoms of enteropathy will further characterize the benefits of PPIs in clinical populations. *Adv Nutr* 2015;6:541–51.

Keywords: enteropathy, inflammatory bowel disease, IBS, agrimedical, plasma protein, immunoglobulin, barrier function, linear growth

Introduction

The gastrointestinal tract functions as both a filter with selective permeability to nutrients and as a defensive barrier that prevents the penetration of foreign entities (1). When exposure to harmful antigens occurs, the subsequent interaction with the local and systemic immune system normally ensues in a well-controlled fashion that maintains gut barrier integrity and establishes appropriate immune activation to provide protection from antigen exposure (1, 2). However, immune dysregulation can occur in the intestinal tract, which causes inappropriate stimulation of immune responses that can lead to inflammatory pathologies (1, 3).

Under normal conditions, intestinal epithelial tight junctions provide an effective barrier against paracellular penetration of luminal antigens (4, 5). However, cytokine-mediated dysfunction of the intestinal mucosal barrier is observed during the active phase of several inflammatory bowel disorders, including inflammatory bowel disease (IBD)[6] (3). Such disease or stress-related states can cause the tight junction barrier to become defective, allowing increased antigenic penetration into underlying intestinal tissues. This results in increased production and secretion of proinflammatory cytokines, including TNF-α, IFN-γ, and ILs. This increase in gastrointestinal permeability allows for

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[6] Abbreviations used: ADFI, average daily feed intake; ADG, average daily growth; D-Glc, D-glucose; G:F, weight gain to feed ratio; IBD, inflammatory bowel disease; IBS-D, diarrhea-predominant irritable bowel syndrome; IC, immunoglobulin concentrate; I-FABP, intestinal fatty acid binding protein; PPI, plasma protein isolate; SBI, serum-derived bovine immunoglobulin/protein isolate; SDP, spray-dried plasma; SEB, *Staphylococcus aureus* enterotoxin B.

translocation and subsequent exposure of microbial antigens to lymphocyte populations, which has been implicated in the symptoms associated with IBD (6–11). In addition, an evaluation of colonic biopsy samples from patients with diarrhea-predominant irritable bowel syndrome (IBS-D) found increased numbers of chronic inflammatory cells accompanied by low-grade inflammation (6, 12). Importantly, such patients also have reduced concentrations of aerobic enteric bacteria relative to healthy individuals (13).

Mammals undergo a weaning process that facilitates the transition from an immunoglobulin-rich liquid diet to a solid diet. The stress of weaning, the dramatic diet change, the immaturity of the immune system, and exposure to environmental microorganisms create an opportunity for shifts in the microbiome in the days postweaning. In animal models as well as in human studies gastrointestinal infections have been shown to initiate inflammation and increases in gut barrier permeability, allowing for translocation of inflammatory microorganisms and other substances. This circular pathway serves as a mechanism through which damaged gut tissue can lead to chronic enteric or systemic disease.

Numerous studies reported that oral administration of plasma protein isolates (PPIs) containing high amounts of immunoglobulins leads to consistent improvements in growth, food intake, and other nutritional variables in various animal species (14–20). Such observations explain the extensive use of PPIs as an animal feed component since the 1980s to improve growth, nutrient utilization, and immunocompetence of domesticated animals (15). The benefits of oral PPIs appear to be related to improvements in intestinal barrier function, microbiota stability, and reductions in gut inflammatory cytokines (14, 16, 17, 21). Many of the studies documenting the benefits of PPIs were conducted in neonatal pigs, which are widely recognized as a leading model to study human nutrition and metabolism, with relevance for agrimedical applications (22). In addition, preliminary clinical studies also demonstrated the safety and utility of dietary supplementation with PPIs in supporting patients with IBS-D (23), HIV-related enteropathy (24), and malnutrition (25, 26). Collectively, these results from both clinical and nonclinical studies indicate that PPI preparations have the potential to provide for distinctive nutritional requirements that are unique to support patients with various forms of intestinal enteropathy (e.g., irritable bowel syndrome, IBD, infantile environmental enteropathy, HIV infection).

Here we report on a meta-analysis designed to determine whether dietary PPIs are effective in improving the nutritional status of animals after experimental challenge with microbial pathogens or toxins known to induce a compromised, inflammatory state. For the purposes of this analysis, dietary animal PPIs were defined as a collection of compounds derived from plasma or serum, including spray-dried bovine plasma, spray-dried porcine plasma, bovine and porcine immunoglobulin concentrate (IC), and bovine serum concentrate. The aim of the current analysis was to evaluate the effects of PPIs on growth, feed intake, and the efficiency of converting feed into muscle mass across a number of domesticated species challenged with microbial pathogens or toxins to determine whether evidence exists to support the use of PPIs as a nutritional supplement in clinical populations experiencing inflammatory bowel disorders.

Methods

Objectives. The objectives of this meta-analysis were to evaluate *1*) the effects of PPIs on weight gain and feed intake in animals with intestinal inflammatory conditions caused by oral challenge with specific microbial pathogens or bacterial toxins and *2*) the implications of such findings for patients with enteropathy and potential outcomes to consider in future clinical studies with PPIs.

Search strategy/study selection. A study selection template was designed to consider which articles were to be included in the meta-analysis. The primary requirement was inclusion of a published summary of the mean effect, sample size used, type and components of the PPI, and associated SDs (or SEs). MEDLINE, EMBASE, and PubMed were searched from 1980 through August 2012. The following terms and keywords were used: ("spray-dried" OR "spray dried") AND (porcine OR bovine OR ovine OR animal) AND (serum OR plasma) as well as ("bovine serum protein") OR ("porcine serum protein") OR ("animal serum protein") AND (growth or performance). Randomized controlled studies of spray-dried bovine and/or porcine plasma reporting outcomes of average daily growth (ADG), average daily feed intake (ADFI), and gain to feed ratio (G:F) were eligible. In addition, a keyword search strategy was used for the period between 1 January 1990 and 31 August 2012 to capture relevant articles that may not have been indexed under the terms used above. These included "serum, diet, performance, pig, porcine, bovine, animal protein, plasma, and blood meal." A manual search of the bibliographies of all accepted studies and of review articles published in the previous 2 y was also performed to ensure complete literature retrieval. Each article obtained through this process was evaluated to determine whether experimental manipulation included exposure to an enteric pathogen or physiologic challenge.

Twenty-nine articles retrieved through this process were considered. Subjects included pigs, cattle, poultry, and rodents. Studies must have been randomized, placebo-controlled studies published in peer-reviewed journals and published abstracts were not included. Whenever results from multiple independent trials were presented in the same publication, each study was considered as an independent experiment and data from these experiments were included as separate entries under the same publication reference. One author (RK) independently reviewed and assessed trials for match with inclusion criteria. After excluding trials that did not fit the criteria, a total of 31 independent experiments or trials from the 29 publications were considered suitable for inclusion in this meta-analysis.

Data extraction and risk of bias. Once the list of articles from published sources was established, we developed a data extraction file from the articles using a spreadsheet. The principal target estimates were mean effect of treatment, mean of the control, and associated sample sizes and SDs and/or SEs. Initial review of candidate articles revealed a high degree of variability in study designs, particularly the time period reported for postchallenge data collection. Consequently, the primary requirements for selection were amended to include a requirement for 14-d postchallenge data collection, which was the most common data collection period represented in the 29 articles. Data collection for ≤10 d was viewed as too short a period of time to assess intervention impact, and studies with data collection periods >14 d varied considerably. All data were hand-entered from published articles and verified to ensure that there were no entry errors. Table 1 defines the variables sought, their detailed descriptions, and any assumptions and modifications made to them in the process of extraction. We performed standard assessments of bias, i.e., a funnel plot of the SE vs. effect size to graphically evaluate any bias. These funnel plots were generated for each of the 3 variables considered (ADG, ADFI, and G:F). In combination with the I^2 statistic, we used the Begg-Mazumdar and Egger tests to assess any publication bias.

TABLE 1 Study outcomes selected for analysis[1]

Variable	Units	Measures/ estimates extracted	Type of design
ADG	g or kg	Mean, *n*, SE/SD	Randomized controlled trial
ADFI	g or kg	Mean, *n*, SE/SD	Randomized controlled trial
G:F	g/kg or g/g	Mean, *n*, SE/SD	Randomized controlled trial

[1] ADFI, average daily feed intake; ADG, average daily growth; G:F, gain to feed ratio.

Statistical analysis. Individual study data were pooled by using the method of moments to estimate the variance between studies (DerSimonian and Laird method) (27). This method does not make any assumptions about the distribution of random effects. The derived effect sizes were combined by using a random-effects model assuming that there was a random effect due to the published studies themselves. In other words, the variability estimate incorporated both within-study variance as well as between-studies variance. This estimate of total variance presents a more robust picture of the true variability of the pooled effect size. Furthermore, heterogeneity was considered to be moderate to high, necessitating a random-effects approach. We considered the I^2 test to assess heterogeneity using the following threshold intervals: ≤90% as moderate heterogeneity and >90% as high heterogeneity. All computations used StatsDirect software version 2.7.9 (StatsDirect Ltd, Altrincham, United Kingdom) to generate the pooled estimates and forest plots of effect sizes, bias estimates, and the measures of consistency such as I^2 and Q.

Results

Overview of included studies. **Table 2** provides a summary breakdown of all the studies considered and reasons for rejecting studies for inclusion in this meta-analysis. As mentioned previously, 2 publications (28, 29) reported on >1 independent experiment (2 each) that used pigs. In these cases, we considered each independent experiment as a separate study and used the data as such in the meta-analysis. As shown in Table 2, 23 of the 29 publications considered for inclusion in this meta-analysis used pigs. However, data from only 10 of these 23 publications could be included in the analysis because 9 studies did not report day 14 data and 10 studies reported no required growth data. The 10 studies that did not report growth study data were used only in selected figures and graphs to describe their study findings. **Table 3** presents the disposition of each study that was reviewed for inclusion in this meta-analysis, along with the microbial agents or toxins, such as lipopolysaccharide (LPS) and *Staphylococcus aureus* enterotoxin B (SEB), which were used in the challenge experiments to induce intestinal infection and inflammation. In total, data from 10 of the 29 studies were included in the analysis (9 studies in pigs, 1 study in turkeys). The same set of outcome measures from

each publication was used to collect data. We did not perform any modifications to these outcome measures and used the data as extracted from the articles with 1 exception. Several articles we reviewed listed the SEM from the ANOVA rather than the SD. Therefore, we used the associated sample sizes to derive an estimate of the SD by using the following formula: SD (σ) = SE $\times \sqrt{n}$. Sample sizes of individual studies varied between 4 and 24, with only 2 of the 11 studies considered having a sample size <10.

Detailed statistics collected from each of the publications considered as well as the effect size estimates including 95% CIs are summarized for ADG (**Table 4**), ADFI (**Table 5**), and G:F (**Table 6**). Corresponding effect sizes and bias assessment forest plots are shown in panels A and B, respectively, in **Figure 1** for ADG, **Figure 2** for ADFI, and **Figure 3** for G:F.

Treatment effect. The pooled effect size for ADG was 0.39 g/d, with the 95% CI between 0.11 and 0.68 (**Table 7**), indicating that the PPI causes a significant increase in ADG over 14 d postchallenge. The pooled effect size for ADFI was 0.36 g/d, with the 95% CI between 0.12 and 0.60 (Table 7), indicating that the PPI causes a significant increase in the ADFI over 14 d postchallenge. Finally, the pooled effect size for G:F was 0.64, with the 95% CI between 0.06 and 1.22 (Table 7), indicating that the pooled effect was positive in favor of the PPI.

Q is the weighted sum of squares on a standardized scale. As a standard score it can be compared with the expected weighted sum of squares to yield an estimate of the null and excess variance. A useful property of Q is that it tests the null hypothesis that all studies used in the meta-analysis share a common effect size. Therefore, a significant P value obtained for Q provides evidence that the true effects vary. However, Borenstein et al. (27) suggested that a nonsignificant P value cannot be taken as evidence that the effect sizes are consistent. Several other factors could contribute to a larger P value, such as low power, small number of studies, larger within-study variance, and substantial between-studies variance. In summary, the purpose of the test for Q is to assess the viability of the null hypothesis rather than to provide an estimate of the magnitude of the true variance.

I^2 is the proportion of the observed variance that is real rather than spurious. It is not dependent on the scale of the effects size or on the number of studies used in the meta-analysis. The small P values (<0.05) associated with the Q-statistic suggest that the null hypothesis (i.e., that the true heterogeneity is really zero) should be rejected and show that there is significant heterogeneity present in the

TABLE 2 Disposition of studies identified during the literature search

Species	Day 14 data available		Day 14 data not available		No data (graphics only)	
	Publications	Experiments	Publications	Experiments	Publications	Experiments
Cows	0	0	1	1	3	3
Pigs	9	11	8	8	6	6
Rats	0	0	0	0	1	1
Turkeys	1	1	0	0	0	0
Total	10	12	9	9	10	10

TABLE 3 Animal species and experimental challenge used in various studies[1]

Authors, year	Reference	Species	Challenge
Included in meta-analysis			
Bosi et al., 2001	(30)	Pigs	*E. coli* K88
Bosi et al., 2004	(16)	Pigs	*E. coli* K88
Campbell and Borg, 2000	(28)	Pigs	*E. coli* K88 O148
Campbell et al., 2004	(18)	Turkeys	*Pasteurella multocida*
Escobar et al., 2006	(31)	Pigs	PRRSV
Owusu-Asiedu et al., 2002	(33)	Pigs	ETEC
Owusu-Asiedu et al., 2003	(34)	Pigs	*E. coli* K88
Torrallardona et al., 2003	(35)	Pigs	*E. coli* K99
Torrallardona et al., 2007	(29)	Pigs	*E. coli* K99
Van Dijk et al., 2002	(36)	Pigs	*E. coli* K82 O139
Excluded from meta-analysis			
No day 14 postchallenge data were collected			
Arthington et al., 2002	(37)	Cows	Coronavirus
Gaines et al., 2003	(32)	Pigs	LPS
Carroll et al., 2003	(38)	Pigs	LPS
Dritz et al., 1996	(39)	Pigs	LPS
Frank et al. 2003	(40)	Pigs	LPS
Owusu Asiedu et al., 2003	(41)	Pigs	ETEC
Polo et al., 2005	(42)	Pigs	PRRSV
Yi et al., 2005	(43)	Pigs	*E. coli* K88
No performance measures were collected			
Corl et al., 2007	(44)	Pigs	Rotavirus
Hunt et al., 2002	(45)	Calves	*Cryptosporidium parvum*
Niewold et al., 2007	(46)	Pigs	*E. coli* ETEC
Nollet et al., 1999	(47)	Pigs	*E. coli* K85b
Pérez-Bosque et al., 2004	(48)	Rats	SEB
Pithua et al., 2009	(49)	Calves	*Mycobacterium avium*
Pujols et al., 2008	(50)	Pigs	PRRSV
Pujols et al., 2011	(51)	Pigs	PRRSV
Quigley and Drew 2000	(52)	Calves	*E. coli*
Touchette et al., 2002	(53)	Pigs	LPS

[1] *E. coli*, *Escherichia coli*; ETEC, enterotoxigenic *Escherichia coli*; PRRSV, porcine reproductive and respiratory syndrome virus; SEB, *Staphylococcus aureus* enterotoxin B.

overall data (Table 7). Furthermore, given this conclusion, the I^2 estimates of ~65% (95% CI: 51.7%, 72.9%) for the 3 analyzed variables show that low to moderate variability exists in the results.

Publication bias. The 2 commonly used tests for publication bias are the Begg-Mazumdar test of Kendall's rank correlation coefficient (τ_b; a nonparametric test) and the Egger test for publication bias (a parametric test) (27). We believe the use of the Begg-Mazumdar test is more appropriate because this test used rank correlation coefficient rather than the ordinary product moment correlation. Ordinary product moment correlation assumes normal distributions, which are unlikely in these situations. Therefore, considering the Begg-Mazumdar test, it can be seen that the publication bias is not significant for all the variables considered in this meta-analysis. It should be noted that the G:F has low power due to the small sample size and high variability in the results (Table 7).

TABLE 4 Average daily growth of study animals fed PPIs from individual experiments[1]

Authors, year	Reference	Species	Treated		Control	
			Mean ± SD, g/d	*n*	Mean ± SD, g/d	*n*
Bosi et al., 2001	(30)	Pigs	100.0 ± 79.7	12	169.0 ± 79.7	12
Bosi et al., 2004	(16)	Pigs	164.0 ± 62.4	12	148.0 ± 62.4	12
Campbell and Borg, 2000	(28)	Pigs	99.8 ± 159.4	24	100.5 ± 159.4	24
Campbell and Borg, 2000	(28)	Pigs	112.2 ± 80.4	24	94.0 ± 80.4	24
Campbell et al., 2004	(18)	Turkeys	134.9 ± 22.7	17	109.5 ± 24.0	19
Escobar et al., 2006	(31)	Pigs	285.0 ± 100.0	16	273.0 ± 100.0	16
Owusu-Asiedu et al., 2002	(33)	Pigs	127.4 ± 27.9	24	102.4 ± 27.9	24
Owusu-Asiedu et al., 2003	(34)	Pigs	156.6 ± 64.3	15	100.9 ± 64.3	15
Torrallardona et al., 2003	(35)	Pigs	211.5 ± 82.8	16	157.5 ± 82.8	16
Torrallardona et al., 2007	(29)	Pigs	142.0 ± 86.8	16	128.0 ± 86.8	16
Torrallardona et al., 2007	(29)	Pigs	223.0 ± 81.2	16	173.0 ± 81.2	16
Van Dijk et al., 2002	(36)	Pigs	42.0 ± 44.4	4	−47.0 ± 44.4	4

[1] PPI, plasma protein isolate.

TABLE 5 Average daily feed intake by study animals given PPIs from individual experiments[1]

Authors, year	Reference	Species	Treated Mean ± SD, g/d	n	Control Mean ± SD, g/d	n
Bosi et al., 2001	(30)	Pigs	251.0 ± 39.1	12	226.0 ± 39.1	12
Bosi et al., 2004	(16)	Pigs	197.0 ± 20.1	12	186.0 ± 20.1	12
Campbell and Borg, 2000	(28)	Pigs	206.6 ± 47.1	48	192.1 ± 47.1	48
Campbell and Borg, 2000	(28)	Pigs	251.1 ± 78.3	48	226.1 ± 78.3	48
Campbell et al., 2004	(18)	Turkeys	229.0 ± 131.5	17	219.6 ± 139.1	19
Escobar et al., 2006	(31)	Pigs	449.0 ± 76.00	16	507.0 ± 76.0	16
Owusu-Asiedu et al., 2002	(33)	Pigs	185.3 ± 70.5	24	152.0 ± 70.5	24
Owusu-Asiedu et al., 2003	(34)	Pigs	213.2 ± 59.3	15	141.1 ± 59.3	15
Torrallardona et al., 2003	(35)	Pigs	277.0 ± 43.2	16	262.5 ± 43.2	16
Torrallardona et al., 2007	(29)	Pigs	230.0 ± 73.6	16	196.0 ± 73.6	16
Torrallardona et al., 2007	(29)	Pigs	301.0 ± 90.8	16	236.0 ± 90.8	16

[1] PPI, plasma protein isolate.

Another way to assess publication bias is to consider the bias assessment funnel plots (shown in Figures 1–3 for ADG, ADFI, and G:F, respectively). A clear pattern observed in all 3 plots indicates that there does not seem to be any missing studies in any of these cases in that all of the studies are concentrated on 1 side of the x axis indicating potential bias. These plots suggest that there is considerable heterogeneity, shown by the points outside the lines, but there is nothing to suggest that there are missing studies.

Discussion

Spray-dried animal plasma in a variety of formulations has been incorporated in the diets of weanling piglets for many years with demonstrable improvements in weight gain and health (15, 19, 54, 55). In this report, we demonstrated a strong association between dietary PPI preparations and improved weight gain and feed intake in weanling animals exposed to experimental challenge. A pooled estimate from 12 published prospective studies suggests that dietary supplementation with PPIs produces significant increases in daily weight gain and feed intake in pigs and turkeys during a 14-d period after experimental challenge.

Agricultural PPI products are prepared primarily from the blood of cattle or pigs with the use of hygienic collection and processing procedures that include addition of anticoagulants, centrifugation to remove RBCs, concentration by filtration or ultrafiltration methods, and spray-drying to create a plasma protein powder. During the spray-drying process the plasma proteins are exposed to high temperatures for a very short period of time to avoid denaturation of proteins and to preserve biological activity (20, 56). The benefits of feeding PPIs has been attributed largely to the immunoglobulin content and include improvements in appetite, weight gain, intestinal growth, and gut barrier function in a number of intestinal disorders (15, 19, 54, 57). A variation of PPI products, serum-derived bovine immunoglobulin/protein isolate (SBI), has been specially formulated to increase protein content and reduce amounts of albumin and fibrinogen, resulting in proportionally higher amounts of immunoglobulins. Plasma protein products used for animal feed typically contain >80% protein and ~15% immunoglobulins (mainly IgG) on a weight basis, whereas SBI preparations are manufactured according to FDA current Good Manufacturing Practices and contain ~92% protein with >50% IgG and high amounts of essential amino acids. SBI has been extensively studied in animal models and was recently found to be safe and effective in the management of enteropathy associated with IBS-D and HIV infection (23, 58).

A large number of studies have evaluated the effects of PPI preparations in weanling animals not subjected to experimental challenge. The overall health of animals during weaning is considered to be compromised due to the existence of various stress factors, including immature intestinal enzymes and other digestive secretions, discontinued access to beneficial factors in colostrum and early milk, and developmental changes during maturation of the intestinal microbiota and immune system. Jiang et al. (14) evaluated growth performance in piglets after pair-feeding a diet containing soy protein or PPIs for 24 d. Protein intake was similar among groups, although the rate of weight gain and protein conversion efficiency was significantly higher in the PPI group, especially during early weaning. Pigs fed PPIs had

TABLE 6 Gain to feed ratio for study animals given PPIs from individual experiments[1]

Authors, year	Reference	Species	Treated Mean ± SD[2]	n	Control Mean ± SD[2]	n
Campbell et al., 2004	(18)	Turkeys	0.58 ± 0.1	17	0.51 ± 0.1	19
Escobar et al., 2006	(31)	Pigs	0.62 ± 136.0	16	0.52 ± 136.0	16
Owusu-Asiedu et al., 2002	(33)	Pigs	0.69 ± 118.6	24	0.68 ± 118.6	24
Owusu-Asiedu et al., 2003	(34)	Pigs	0.74 ± 0.1	15	0.72 ± 0.1	15
Torrallardona et al., 2003	(35)	Pigs	0.77 ± 0.3	16	0.57 ± 0.3	16

[1] PPI, plasma protein isolate.
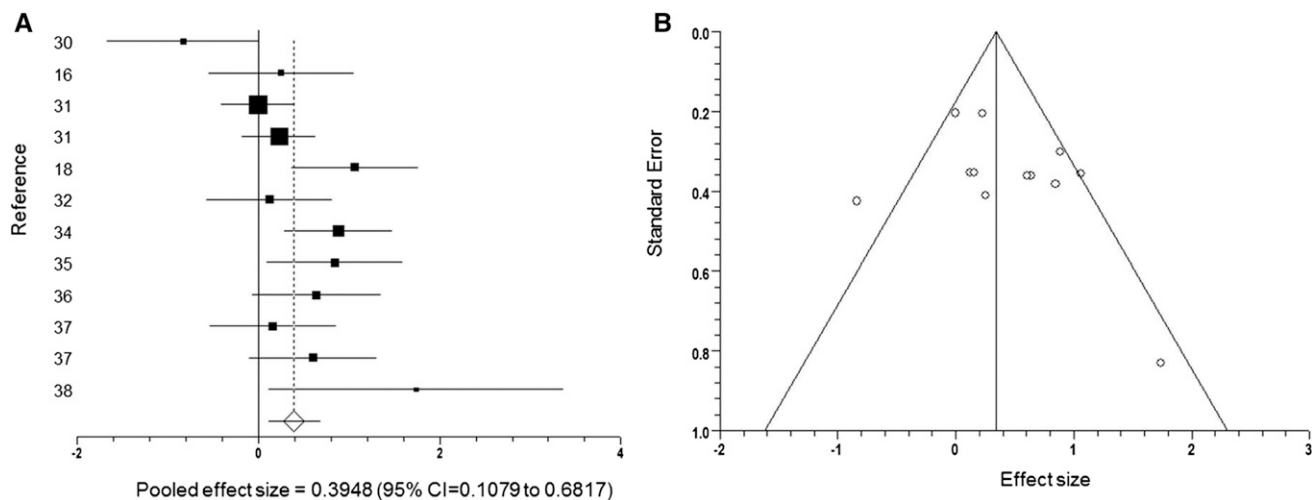[2] Ratio of weight gain (g) to feed (g).

FIGURE 1 Forest plots summarizing average daily growth data. (A) Effect size meta-analysis plot (random effects). (B) Bias assessment plot.

improvements in body weight and protein mass with no difference in fat mass, suggesting a higher efficiency of dietary protein utilization for lean tissue growth. Feeding PPIs reduced the circulating concentrations of urea, arginine, citrulline, and ornithine, suggesting a reduction in the catabolism of amino acids to urea and increased availability of dietary amino acids for lean tissue mass. In addition, there were significant increases in bone mineral content and bone mineral density in PPI-fed pigs compared with soy protein-fed pigs. Pierce et al. (54) conducted several experiments to evaluate the growth and feed intake of weaned piglets fed porcine PPIs, bovine PPIs, or different molecular-weight fractions of PPIs. These investigators reported that both porcine and bovine PPIs enhanced growth rate and feed intake of weaned piglets, whereas the IgG fractions appeared to stimulate growth performance that was comparable to that for intact PPIs and superior to that for the albumin or low-molecular-weight

fractions of PPIs. These data suggest that a distinct nutritional role may exist for the IgG-rich fraction of PPIs to support growth performance. Reviews were also published in recent years that document the benefits of PPI supplementation in weanling animals. Torrallardona (19) summarized the results from 75 trials involving >12,000 pigs that evaluated the feeding and nutritive benefits of PPIs from a variety of sources, in both healthy and challenged piglets. Most studies showed improvements in caloric intake, growth and metabolism, and utilization of feed nutrients after PPI supplementation in healthy piglets. In addition, improvements in weight gain and feed intake in piglets were found to be consistently greater with PPIs than with feeding comparable amounts of other high-quality protein sources (e.g., meat extracts, soy, pea, potato, skimmed milk, whey, and fishmeal). Although their review was not designed to systematically review the effects of PPIs in piglets exposed to
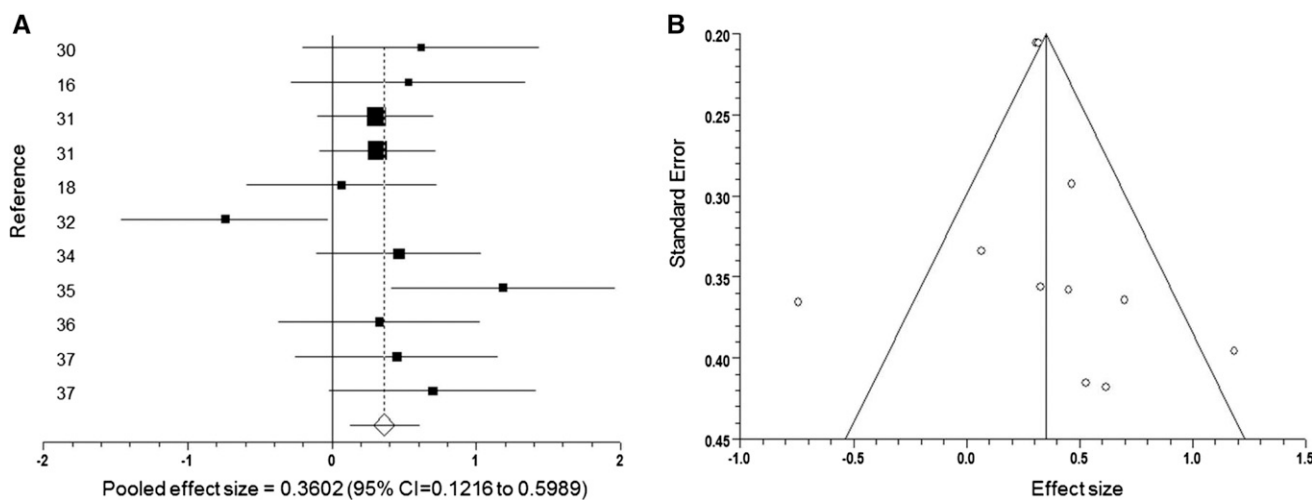

FIGURE 2 Forest plots summarizing average daily feed intake data. (A) Effect size meta-analysis plot (random effects). (B) Bias assessment plot.
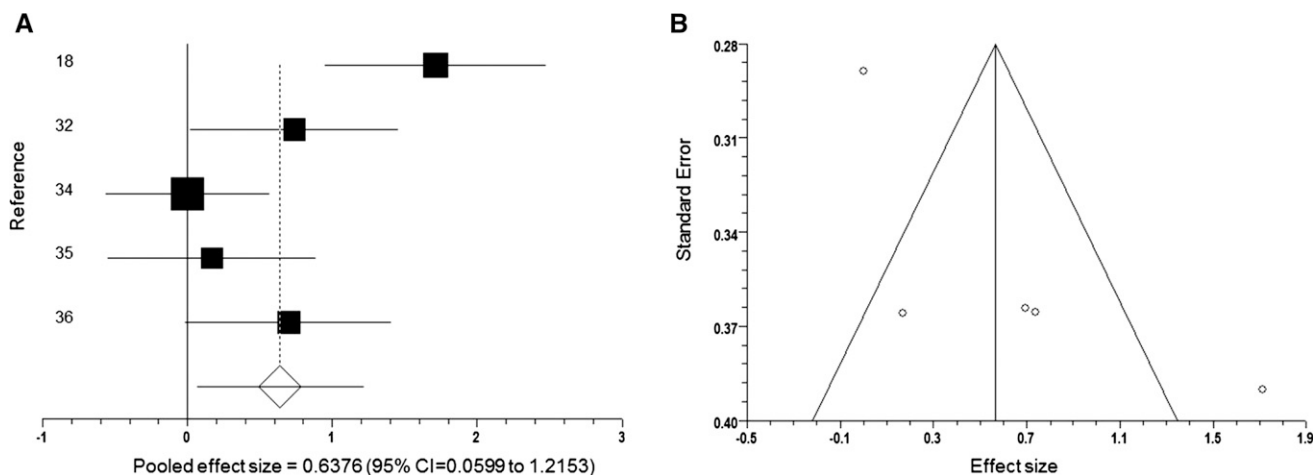
FIGURE 3 Forest plots summarizing gain vs. feed ratio data. (A) Effect size meta-analysis plot (random effects). (B) Bias assessment plot.

an experimental challenge, it was clear that immunoglobulin-enriched protein formulations had similar effects on daily G:F ratio when compared with studies involving healthy piglets.

The findings of our meta-analysis are consistent with results reported from studies of PPIs in healthy weaning animals, and extend the review by Torallardonna (19). We demonstrated a strong association between dietary administration of PPIs and weight gain and feed intake in animals exposed to experimental challenge. Although a number of studies that evaluated PPIs in challenged animals were not included in this systematic review, several reported consistent findings that shed light on the potential mechanism of action of PPIs. For example, Corl et al. (44) evaluated PPI- and soy protein-based diets in rotavirus-infected and noninfected weanling piglets to assess effects on acute rotavirus-induced intestinal damage or improved recovery. Infected, PPI-fed piglets maintained growth rates similar to those of noninfected piglets and showed no clinical signs of diarrhea during the first 3 d of infection, whereas soy protein–fed piglets experienced reduced weight gains and diarrhea. Yi et al. (43) did not report 14-d growth data but found that spray-dried plasma (SDP) mitigated villous atrophy and intestinal morphology impairment in weaned piglets after *Escherichia coli* challenge. In a study reported by Bhandari et al. (59), piglets fed PPIs and challenged with *E. coli* had lower diarrhea scores ($P < 0.05$) and increased survival ($P < 0.05$) compared with pigs fed a control diet, but they did not show significant improvements in ADG, ADFI, or G:F by day 7 postinfection. Pérez-Bosque et al. (60) did

not study growth but evaluated the potential modulatory effects of dietary spray-dried PPI or ICs on intestinal barrier function in rats after exposure to SEB. In this study, PPI and IC supplementation reduced the effects of SEB on dextran and horseradish peroxidase paracellular flux, suggesting possible maintenance of intestinal permeability to prevent the passage of microbial and food antigens to the interstitial space and inflammation. In a series of other studies, Pérez-Bosque et al. (48, 61–63) examined whether PPI and IC supplementation could modulate cytokine expression and inflammatory mediators in rats challenged with SEB. Both PPI and IC diets reduced intestinal water secretion vs. control diets, thus improving nutrient absorption and electrolyte homeostasis (48). Compared with the control diet, PPIs and ICs also reduced SEB-induced increases in lymphocyte populations with specific functions in inflammatory states. The PPI diet prevented major activation of CD4+ cells induced by SEB, indicating that rats fed PPIs did not develop the same degree of activation of T helper cells, and prevented the SEB-induced increase in the γδ-T lymphocytes. Several other studies among those that were excluded from this analysis also showed positive effects of feeding PPIs (41, 45, 46).

Pérez-Bosque et al. (62) also evaluated the potential modulatory effects of diets containing added PPIs or ICs on lamina propria and intraepithelial lymphocytes (diffuse gut-associated lymphoid tissues) in the same model of mild intestinal inflammation induced by intraperitoneal administration of SEB. In lamina propria, SEB increased the

TABLE 7 Measures of pooled effect sizes, consistency estimates, and publication bias[1]

Variable	Pooled effect size (95% CI)	Consistency estimates		Publication bias	
		Q, df (P)	I^2, % (95% CI)	Begg-Mazumdar, Kendall's τ_b (P)	Egger bias (95% CI) [P]
ADG (g/d)	0.39 (0.11, 0.68)	24.59, 11 (0.0105)	55.3 (0.0, 75.1)	0.21 (0.3807)	1.66 (−1.33, 4.66) [0.2446]
ADFI (g/d)	0.36 (0.12, 0.60)	15.92, 10 (0.1020)	37.2 (0.0, 67.8)	0.42 (0.0866)	0.73 (−2.67, 4.13) [0.6403]
G:F	0.64 (0.06, 1.22)	14.00, 4 (0.0073)	71.4 (0.0, 86.7)	0.60 (0.2333)	11.67 (−6.31, 29.65) [0.4646]

[1] ADFI, average daily feed intake; ADG, average daily growth; G:F, gain to feed ratio.

cytotoxic lymphocyte populations of γδ-T cells by 38%, NK cells by 59%, and the number of activated T lymphocytes by 148%. Both PPIs and ICs significantly decreased the effects of SEB on these lymphocyte subsets. In the epithelium, SEB induced a 117% increase in intraepithelial activated lymphocytes that was also significantly reduced by PPI supplementation (although not IC supplementation). The effects of plasma supplements on intestinal lymphocyte populations suggest that oral PPIs can modulate the degree of activation of diffuse gut-associated lymphoid tissues. In a follow-up study, Pérez-Bosque et al. (61) found that SDP inhibited the increase in IFN-γ, IL-6, and leukotriene B4 (LTB4) induced by SEB. SDP supplementation increased IL-10 and mature TGF-β concentrations in intestinal mucosa from both SEB-challenged and control animals. Both immunoglobulin supplements were effective at preventing the SEB-induced increase in proinflammatory to anti-inflammatory cytokine ratios in Peyer's patches, mucosa, and serum. Other studies reported similar effects of dietary PPIs in terms of decreasing cytokine expression or T cell activation in animal models of colitis (64, 65). Collectively, these results support the hypothesis that a diet containing PPIs can play a role in the modulation of the immune response by limiting the immune activation that can compromise gut barrier function and ultimately the utilization of food energy.

Intestinal inflammation can lead to alterations to gut barrier function and impairments in nutrient absorption. Various studies evaluated whether such an effect can be mitigated by PPI supplementation. Garriga et al. (66) evaluated the impact of PPI supplementation on intestinal transport of D-glucose (D-Glc) and 3 essential amino acids in a rat model of intestinal inflammation induced by SEB. The administration of SEB significantly reduced D-Glc transport and expression of D-Glc transporters in intestinal brush border membrane vesicles. Dietary spray-dried PPIs increased D-Glc transport by 10% compared with the SEB group. Changes in D-Glc transport due to SEB and to PPIs were correlated with changes in the number of sodium-glucose linked transporter 1 (SGLT1) transporters present in the brush border membrane. It was estimated that PPIs included in the diet increased glucose absorption by 8–9% in rats challenged with SEB during the interdigestive periods. Collectively, these results suggest that dietary administration of PPIs can help maintain intestinal homeostasis by reducing gut permeability and inhibiting local inflammation, thereby decreasing passage of microbial and food antigens to the interstitial space and supporting nutrient absorption.

It is also interesting that the administration of PPIs or SBI showed similar benefits in human trials. Bovine-derived immunoglobulin from colostrum with activity for *Clostridium difficile* when administered orally to healthy volunteers showed reactivity for *C. difficile* in ileal contents (67). Isolation of bovine anti-*C. difficile* antibodies from feces after oral administration of colostrum was also shown to react with the bacterium (68). Bovine-derived immunoglobulins have also been shown in AIDS patients with chronic, severe diarrhea caused by *Cryptosporidum parvum* to reduce the incidence and severity of the enteropathy (69). Infants suffering from diarrhea caused by enteropathogenic *E. coli* and given oral milk-derived immunoglobulins from lactating cows for 10 d led to stool cultures that were negative for enteropathogenic *E. coli* in >80% of cases (70). In nonclinical and clinical studies, anti-rotavirus antibodies from milk or plasma protein given orally were effective in maintaining intestinal health and protecting against rotavirus infection (44, 71, 72).

Nutritional therapy with SBI also has provided management of IBS-D and HIV-associated enteropathy in humans. Wilson et al. (23) conducted a randomized, double-blind, placebo-controlled, single-site study in which subjects with IBS-D were administered for 6 wk with either 10 g/d SBI, 5 g/d SBI + 5 g/d soy protein isolate, or placebo (10 g/d soy protein isolate) as a dietary intervention. Subjects in the 10-g/d SBI group showed significant reductions in the number of days with symptoms from week 2 to week 6 for abdominal pain ($P = 0.01$), flatulence ($P = 0.01$), bloating ($P = 0.05$), loose stools ($P = 0.01$), urgency ($P = 0.05$), and any symptom ($P = 0.01$). Subjects in the 5-g/d SBI group showed reductions from week 2 to week 6 in the number of days with flatulence ($P = 0.035$), incomplete evacuation ($P = 0.05$), and any symptom ($P = 0.01$). Greater improvements in loose stools, hard stools, flatulence, and incomplete evacuation also were achieved by SBI administration, with no therapy-related adverse events reported. In an open-label study by Asmuth et al. (58), 8 subjects with HIV-associated enteropathy showed improvements in gastrointestinal symptoms with reduced bowel movements per day ($P < 0.008$) and improvements in stool consistency ($P < 0.008$). Seven of the 8 subjects also showed increased uptake of D-xylose, suggesting improved absorption of nutrients. A marker for enterocyte damage, intestinal FA protein (I-FABP), initially increased in 7 of 8 subjects after 8 wk ($P = 0.039$), but then fell below baseline in 4 of 5 subjects who continued taking SBI for 40 additional weeks ($P = 0.12$), suggesting that inflammation-based destruction of enterocytes had been ameliorated. In addition, SBI significantly increased mucosal CD4+ lymphocyte densities over 8 wk, but had no effect on circulating CD4+ counts in this small sample size, and caused a decrease in inflammation-induced tissue remodeling matrix metalloproteinases, suggesting a dampening of inflammation- and tissue-specific remodeling in the intestine. Collectively, data from these studies support the hypothesis that oral SBI can play a role in helping to restore intestinal immune balance.

Limitations of the study. We conducted a meta-analysis of ADG, ADFI, and G:F outcomes over a 14-d period postchallenge with either LPS or enteric pathogens in 2 different animal species (9 studies in piglets, 1 study in turkeys) to evaluate the effects and extrapolate these findings toward possible beneficial effects in humans. All of the literature studies compiled in this meta-analysis used robust, randomized, and controlled designs and used standard statistical principles in the design, conduct, and analysis of these experiments. The effects observed in the 9 pig studies and the 1 study involving turkeys were consistently positive and support the inference that PPIs may

produce similar beneficial effects in humans. A key limitation of this study was the need to exclude a number of studies for a variety of reasons and to limit the analysis to studies reporting 14-d results. Studies were excluded because they *1*) had multiple diet changes, *2*) did not report performance data, or *3*) reported data after as little as 7 d or as long as 48 d after treatment with PPIs. Additional studies are needed to better define the role of oral immunoglobulins in maintaining immune balance and strengthening gut barrier function in human gastrointestinal disorders such as Crohn disease, ulcerative colitis, and irritable bowel syndrome.

Conclusions

Studies with PPIs have shown consistently positive effects across multiple species on growth, food intake, and nutritional status in animal populations with inflammation. The meta-analysis reported here involving 10 publications and 12 experiments further documents that PPI preparations are beneficial in supporting weight gain in animals with compromised intestinal function (e.g., infection, inflammatory conditions). Results from these studies support a role for PPI preparations in maintaining intestinal immune balance, supporting gut barrier function, and improving nutrient absorption, which may have implications for helping the nutritional status of patients with compromised intestinal function due to various disease states. Further studies are needed to assess the effective dose amounts of PPIs for the dietary management of various intestinal conditions.

Acknowledgments

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