RESEARCH REPORT
ANIMAL SCIENCE

Title: Critical Review of Functional Animal Proteins – **NPB #04-142**

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Date submitted: July 26, 2006

Abstract:
We have reviewed the scientific literature on the use of functional animal proteins in pig diets for the purpose of improving pig health and productive performance. Our focus is on physiological effects beyond provision of bioavailable nutrients. We address both the proposed mechanisms of action and the empirical data on practical results. Products considered are spray-dried plasma, dried porcine solubles, milk proteins and egg products. Spray-dried plasma continues to increase growth rate dramatically, on average more than 20%, when fed for a short time after weaning. There appears to be a small reduction in growth rate after plasma is removed from the diet, but some advantage is retained. Recent data suggest plasma also provides protection against enteric disease. The limited data on dried porcine solubles are encouraging, but conclusions remain tentative. Among milk proteins, those in whey appear to provide modest benefits. It is not yet clear whether conventional egg products provide physiological benefits, but they are clearly less powerful than is spray-dried plasma. Appropriately-targeted immune egg products can provide impressive protection against enteric disease. In summary, spray-dried plasma and immune egg products provide dramatic benefits, whey proteins provide modest ones, and the benefits of dried porcine solubles and of conventional egg products remain unclear.

Introduction:
The Non-Antimicrobial Production Enhancers (NAPES) Committee of the National Pork Board believes that there is a substantial amount of information on potentially useful feed ingredients in the literature that can be very useful if reviewed critically and thoroughly to summarize the existing knowledge. Thus, the NAPES Committee commissioned several critical reviews of the literature on these feed ingredients. This is one of them, focusing on feed ingredients that contain functional animal proteins.

Objective:
To draw conclusions from the scientific literature about the potential usefulness of several products containing functional animal proteins as non-antimicrobial production enhancers.

Materials & Methods:
For each product or class of products, we searched thoroughly the following bibliographic databases:

- PubMed
- Commonwealth Agricultural Bureaux International (CABI)
- Journal of Animal Science (including abstracts)

We also searched relevant websites and our files, and obtained appropriate papers cited in earlier reviews. We summarized the results across experiments by meta-analyses, using the results cautiously.

Results:
Our complete review accompanies this report.

Discussion:
Thorough discussions of the results for various products are included in the detailed review that accompanies this report.

I. Lay Interpretation:
Spray-dried plasma and immune egg products provide dramatic benefits when added to diets of young weaned pigs. Spray-dried plasma increases growth rate and resistance to enteric infection, while properly-targeted immune egg products markedly improve resistance to disease. Whey proteins provide modest benefits in growth rate. The benefits of dried porcine solubles and of conventional egg products remain unclear.

A Critical Review of Functional Animal Proteins

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Introduction

Animal diets provide bioavailable nutrients, but there is growing appreciation for the notion that certain dietary ingredients can provide as well further benefits in health and productive performance. Among these potent dietary ingredients are certain oligosaccharides and other specific carbohydrates, yeasts and yeast products, direct-fed microbials (probiotics), acidifiers, botanicals, and certain animal products. Interest in such ingredients is intensifying as the industry contemplates the potential enactment of severe limitations on the use of antimicrobials. While it is unlikely that any of these potent dietary ingredients has exactly the same range of activity and potency as antimicrobials, they are often considered “alternatives to antibiotics”.

The purpose of this document is to review the information available on specific animal products that may be incorporated in pig diets, with an emphasis on functional proteins within those products. We consider the following products:

- Plasma products
- Dried porcine solubles
- Milk proteins
- Egg products

Search procedures

We used three search engines (PubMed, CAB, Agricola), using several appropriate keywords for each search. We specifically searched the *Journal of Animal Science*.

Assessment of functional properties

Functional foods are those that provide benefits to the animal beyond provision of bioavailable nutrients. Accordingly, our assessment of whether the dietary ingredients of animal origin under consideration are indeed functional foods has two parts:

- Consideration of the proposed mechanisms of beneficial effects of the animal proteins in question, including both the logic and the evidence supporting those mechanisms;
- Evaluation of the empirical data to determine whether the mechanisms of action result in demonstrable practical benefits.

We used meta-analyses to evaluate the empirical data describing animal responses in disease resistance and in growth performance; results from multiple experiments were tabulated to evaluate the overall dataset. Results of meta-analyses of data in published literature must always be interpreted cautiously because some scientists choose to not publish results that fail to show clear differences between treatments, and this unfortunate policy results in a bias in the published literature. That bias is most obvious when a review of the literature is formalized in a meta-analysis, but it influences any review of published information. The practical result in the present case is that a meta-analysis may exaggerate the value of a novel animal protein source. However, in some cases the situation is more complex. The value of spray-dried plasma, and to a lesser extent of milk proteins, has become generally accepted by the scientific community, so there may now be a perception that showing a positive response to these ingredients does not contribute to knowledge but showing a lack of

response does contribute. That attitude may, to an unknown degree, reverse the expected bias. Further, much of the recent research on practical impacts of spray-dried plasma is not conducted to confirm the value of plasma, but to use it as a standard to which new ingredients are compared. In that case, superiority of the new test ingredient may be considered publishable, adding to the reversal of bias.

Most of the products considered are included in pig diets at levels of several percent of the diet, and thus provide nutriture as well as purported functional properties. These factors complicate evaluation of empirical responses because they require segregation of the responses to nutrients from the responses to other functions. Segregating such factors is never perfect, but we have taken steps to guard against confounding of functional effects with levels of bioavailable nutrients. First, data were summarized only if the experimental diets appeared similar in nutrient content. Second, studies were eliminated in which diets were clearly deficient in some nutrient, as in experiments designed to measure nutrient bioavailability in ingredients. In that case the response to the nutrient would likely overwhelm any response to a functional protein. The purpose is to determine whether there are responses to the ingredients that are not due to provision of bioavailable nutrients.

We used only data from peer-reviewed publications in our meta-analyses. The design of each experiment was assessed carefully to verify that each evaluation of the ingredient in question had an appropriate control treatment, and that the test was not confounded with other factors.

Nearly all of the studies summarized were conducted with young pigs shortly after weaning, because that is the stage of life at which the pig is most vulnerable to external challenges, including diseases. Where data were reported for more than one time period, we chose a short period shortly after weaning to enhance sensitivity of the tests. Specifically, a period as near to 14 days as possible was chosen; fewer data are available for 7 days.

Where an experiment evaluated a series of levels of an ingredient, only the data from the extreme treatments were evaluated unless a specific consideration dictated a different choice. Responses are expressed as percentage changes from the appropriate control treatment to avoid biasing the overall assessment toward the experiments with faster growth, which in some cases was due to older age of the pigs.

Plasma Products

a. Description of products

Blood is collected at slaughter plants, treated with an anticoagulant, and transported while fresh to a processing plant. There the plasma is separated by centrifugation and gently spray-dried.

The product may be further processed to standardize immunoglobulin concentrations, to convert to a physical form that is easier to handle in a feed manufacturing plant, or to make it water-soluble.

It is useful to remember that plasma carries important physiological signals through the body, so it contains a myriad of functional components, although most if not all published research concerning plasma products in animal feeds has focused on plasma proteins.

b. Proposed/demonstrated mechanism of action

The growth promoting properties of spray-dried plasma are well recognized; the recent evidence is summarized in the following section on efficacy. However, the mechanisms underlying this enhancement of animal performance are poorly understood, despite an extensive number of feed trials worldwide by various research groups. The proposed mechanisms generally fall into three categories:

- Increased feed intake
- Protection against disease
- Improved intestinal structure

The simplest explanation for the beneficial effects could be that plasma stimulates the growth rate largely by increasing feed intake (Jiang et al., 2000b). Most studies indeed demonstrate that spray-dried plasma exerts a stimulatory effect on feed intake. This stimulation of feed intake is a beneficial response because inadequate feed consumption is an important factor limiting growth rate during the weaning transition. However, the mechanism driving a possible increase in feed intake is not known. It is of interest to note that while feed is withdrawn routinely for some number of hours from animals prior to

slaughter, surprisingly little attention has been given to possible orexigenic factors in plasma of animals that have been deprived of feed.

The hypothesis that plasma protects pigs from disease challenge is supported by an early study by Coffey and Cromwell (1995), where weanling pigs fed 20% spray-dried plasma from day 0 to 14 after weaning followed by feeding a common diet from days 14 to 28 were used to determine the effect of nursery environment on growth performance. Pigs were housed in either an environmental chamber in new pens (experimental nursery) or a more typical, on-farm, conventional nursery. Pigs in the experimental nursery grew significantly faster than the pigs in the conventional nursery from days 0 to 14 and from days 0 to 28. Growth rate and feed intake were enhanced by feeding plasma to pigs in the conventional nursery, but the responses to plasma were of considerably less magnitude in pigs housed in the experimental nursery (Coffey and Cromwell, 1995). The authors suggest that the presence of a natural pathogenic challenge may play an important role in the magnitude of the neonate's response to dietary plasma (Coffey and Cromwell, 1995). A similar environmental influence on the response to dietary supplementation of plasma in broiler chickens was reported by others (Campbell et al., 2003). In Lewis rats, dietary supplementation of 8% of plasma reduced water content in feces after challenge with a double dose of *Staphylococcus aureus* enterotoxin B (Garriga et al., 2005; Perez-Bosque et al., 2004).

The means through which dietary plasma protects against disease may include direct effects of the exogenous immunoglobulins in plasma, actions of glycoprotein glycans, and immunomodulation.

Perhaps the direct action of the plasma immunoglobulins is the most widely purported mechanism of dietary plasma's role in protection against disease. Plasma immunoglobulins retain biological activity through the spray-drying process (Owusu-Asiedu et al., 2002). Immunoglobulin action is supported by research in both pigs (Pierce et al.,

2005) and mice (Godfredson-Kisic and Johnson, 1997) that showed the growth-promoting properties of dietary plasma lie in a fraction containing high-molecular weight proteins, including immunoglobulins. Fractions of medium or low molecular weight were without detectable benefit. These results must be interpreted cautiously because the high-molecular-weight fraction contained other components in addition to immunoglobulins. Notably, benefits in pigs (Pierce et al., 2005) were obtained from the high-molecular-weight fraction of bovine as well as porcine serum. The animals from which the bovine plasma were obtained would presumably have been exposed to a different repertoire of antigens than were the pigs that consumed the experimental diets. This brings into question the notion that plasma immunoglobulins are primarily responsible for the growth-promoting effects of plasma; this issue has not been adequately addressed.

Glycoprotein glycans from bovine plasma inhibit pathogenic *Escherichia coli* adhesion to bovine erythrocytes, small intestinal mucus and brush border membranes (Nollet et al., 1999). Bovine plasma preparations have been used as adhesion blockers in studies of neonatal colostrum-deprived calves to reduce diarrhea and mortality caused by pathogenic *E. coli*. Bovine plasma preparations lacked specific antibodies against the challenge strains, so Nollet et al. (1999) suggested that the protective effect of plasma could be due to glycoproteins inhibiting the adhesion of pathogenic *E. coli* to intestinal receptors.

The immunomodulatory effects of dietary plasma are complex and not completely understood. Feeding plasma to healthy pigs reduces inflammation, which may be important in increasing both feed intake and growth rate. Specifically, pigs fed diets containing plasma had reduced mRNA expression levels of inflammatory cytokines, including tumor-necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6) and interleukin-8 (IL-8) in adrenal gland, spleen, hypothalamus, pituitary gland, liver and jejunum (Bosi et al., 2004; Touchette et al., 2002) In addition, plasma-supplemented diets reduced

inflammatory cell infiltration in jejunum (Bosi et al., 2004) and reduced the density of activated T helper cells in Peyer's patches (Pérez-Bosque et al., 2004).

A down-regulation of the hypophyseal-pituitary-adrenal (HPA) axis was also observed (Carroll et al., 2002), which the authors attribute to the down-regulation of inflammatory cytokine expression. Specific effects on the HPA axis include reduced expression of hypothalamic corticotrophin-releasing hormone (CRH), reduced expression of the CRH receptor in the pituitary gland, reduced circulating levels of adrenocorticotropic hormone (ACTH) produced by the pituitary gland, and reduced expression of ACTH receptor in the adrenal gland (Carroll et al., 2002). There were no detectable effects on the somatotropic axis, as measured by expression of insulin-like growth factors 1 and 2 (IGF-1 and IGF-2) in liver, muscle and adipose (Matteri et al., 2000).

A stimulatory effect of dietary plasma on immune function of immunologically challenged pigs (Frank et al., 2003; Touchette et al., 2002) stands in sharp contrast to the dampening of inflammation seen in healthy pigs. In pigs fed a diet with plasma, lipopolysaccharide (LPS) challenge did not affect cytokine mRNA expression, whereas LPS reduced expression of TNF-α mRNA in the spleen and IL-1β mRNA in the adrenal gland, spleen, and thymus of pigs fed the diet without plasma (Touchette et al., 2002). In pigs fed the diet with plasma, LPS administration increased serum TNF- α concentrations 150-fold compared to a 60-fold increase for pigs fed the control diet. Similarly, serum interferon-γ (IFN-γ) concentrations increased 110-fold in pigs fed the diet with plasma compared to a 16-fold increase in pigs fed the diet without plasma. In pigs fed the diet with plasma, LPS administration caused serious villous atrophy, whereas LPS had no observable effect on intestinal morphology for pigs fed the diet without plasma.

Collectively, these results indicate that dietary plasma dampens the basal activation of the immune system but heightens the response to immunologic challenge. However,

cellular and molecular mechanisms underlying these immunomodulatory effects remain undefined.

The intestinal structure of pigs changes markedly and predictably after weaning. There is first a reduction in villous height, followed after several days by a marked hyper-regeneration of the villi and deepening of the crypts (Pluske et al., 1996). The initial villous atrophy impairs digestive and absorptive function of the gut, contributing to poor performance after weaning (Van Dijk et al., 2001b). The refereed literature (Jiang et al., 2000b; Van Dijk et al., 2001b) suggests that dietary plasma does not alter these intestinal changes. There were also no effects on lactase, sucrase or maltase enzymatic activities associated with the brush border (Van Dijk et al., 2002b). As noted earlier, plasma causes marked villous atrophy after an LPS challenge, in association with a heightened immune response (Touchette et al., 2002).

Plasma in weanling pig diets decreased the relative growth of the small intestine after 16 days of supplementation, in spite of the lack of effect on mucosal villous height, crypt depth or cell proliferation index, which would explain the reduced intestinal protein and DNA masses in the pigs fed plasma (Jiang et al., 2000b). The authors speculated that the reduced intestinal weight may be associated with the lamina propria rather than the epithelium, and may reflect decreased intestinal inflammation. This suggestion is consistent with the dampening of the immune system of healthy animals by dietary plasma described earlier. The reduced intestinal weight and lamina propria cell density may also reflect a reduction in total microbial numbers in the intestine, consistent with the effects of antimicrobial growth promoters (Collier et al., 2003).

In summary, the mechanisms through which dietary plasma improves growth performance are not completely clear. An increase in feed intake is likely important, although the mechanisms of that increase are unknown. Dietary plasma appears to increase disease resistance, apparently through its immunoglobulin content, its

glycoprotein glycans, and modulation of the immune system. It appears to dampen inflammation in healthy animals, and that effect may be important in increasing both feed intake and growth rate. In contrast, plasma appears to enhance the immune response to a challenge. It appears that dietary plasma does not alter villous height or crypt depth in healthy animals.

c. Efficacy

Van Dijk et al. (2001a) and Coffey and Cromwell (2001), working separately, summarized the available data on the impact of spray-dried plasma on growth rate of young pigs and found increases of, respectively, 27% and 25%. Few management options in swine production produce such dramatic responses. The size of the response has driven the industry to aggressively adopt the use of spray-dried plasma in spite of its price being higher than prices of alternative ingredients. We excluded from our summary (Table 1) the studies reviewed in these two earlier summaries.

As noted earlier, many of the recent studies that involve spray-dried plasma use it as the standard to which other ingredients are compared. There has been an aggressive search for alternatives to plasma, driven by both the cost of plasma and concerns in some circles about the potential for transfer of transmissible spongiform encephalopathies or other diseases through plasma. While partial replacement of plasma by other ingredients has often been successful, the data summarized in Table 1 indicate that complete replacement has not.

Our summary shows an unweighted mean improvement of growth rate of 23%, remarkably similar to the previous estimates (Coffey and Cromwell, 2001; Van Dijk et al., 2001a) although there was no overlap in data. Our summary identified 69 comparisons with positive responses to spray-dried plasma, and only 5 with negative responses. These data indicate that pigs weaned later are at least as responsive to dietary plasma as those weaned early. Further analysis shows the response to plasma is not greatly different when it replaces soybean meal in the diet (21% increase in growth rate) than when it replaces special expensive ingredients targeted specifically for diets of pigs shortly after weaning (25%).

We interpret these results to confirm that spray-dried plasma provides benefits beyond provision of bioavailable nutrients. Previous research has shown a larger response to spray-dried plasma in a more-stressful environment (Coffey and Cromwell, 1995), consistent with specific physiological benefits of plasma.

Additional evidence for efficacy comes from two studies that do not fit into our summary. Addition of a water-soluble product derived from plasma to the drinking water of newly weaned pigs increased ($P<0.01$) their growth rate during the first 4-7 days after weaning in two experiments, with bigger responses when the diet was low in quality (Steidinger et al., 2002). Inclusion of a commercially available product containing porcine immunoglobulins in the liquid diet of colostrum-deprived artificially reared piglets improved their survival ($P<0.01$) and early growth rate ($P<0.05$) in research by Gomez et al. (1998).

There is some evidence of variation in potency among commercial sources of spray-dried plasma (DeRouchey et al., 2004).

There has been concern that the benefits of spray-dried plasma are short-term; that pigs grow more slowly after withdrawal of plasma from the diet than do contemporaries that did not consume plasma. A substantial body of data related to this issue (Table 2) does not support clear conclusions, but it appears likely the following hold:

- That pigs grow at a slower rate after withdrawal of plasma than do controls. The effect is small (2%) and inconsistent (occurred in 26 cases with 18 reversals).
- That the compensation is incomplete. At the end of a compensation period, pigs previously fed plasma retained the advantage (measured as overall growth rate or as final weight) in 36 of 48 cases. Performance was usually measured for periods of only 10 to 28 days after withdrawal of plasma from the diet, so it is possible that further compensation could occur over a longer time. Only one refereed publication was found that reported performance over longer withdrawal periods (102 days; Carlson et al., 2005). In this case, the overall effect of plasma on growth rate in two comparisons was negative; however, it should be noted that the overall effect in this case was negative after 28 days of withdrawal (data not shown), placing this study in the minority (10) of the 48 observations.

Perceptions of the mode of action of spray-dried plasma include enhanced resistance to disease and there now exist a few tests of the impact of plasma on resistance to *E. coli* infection (Table 3). Spray-dried porcine plasma in the diet improved clinical outcomes in 10 of 11 cases in the face of either an imposed challenge or a natural infection. In an additional study, excluded from Table 3 because the doses of plasma were far higher than in the cases listed, plasma appeared to provide some protection from the edema disease caused by an F18 *E. coli* challenge (Nollet et al., 1999). It provided protection against both K88 and F18 strains of pathogenic *E. coli*, consistent with high antibody titers against those surface antigens in the plasma used (Owusu-Asiedu et al., 2002; Owusu-Asiedu et al., 2003a). It should be noted that the control treatment in some cases was a spray-dried animal plasma with low antibody titers against the *E. coli* antigens or the same spray-dried plasma tested, but autoclaved before use (Owusu-Asiedu et al., 2002).

Other investigators (Bosi et al., 2004; Torrallardona et al., 2003) tested the effects of spray-dried plasma in the face of an imposed *E. coli* challenge, but the challenge was not strong enough to cause clinical disease so these studies were included in the summary of performance effects (Table 1).

The protective effect of plasma against other pathogens has not been tested in pigs, but plasma has been reported effective against coronavirus (Arthington et al., 2002) and cryptosporidia (Hunt et al., 2002) in calves and against *Staphylococcus aureus* superantigen B in rats (Garriga et al., 2005; Perez-Bosque et al., 2004).

In summary, there is strong evidence that spray-dried plasma provides benefits beyond provision of bioavailable nutrients. The impacts appear in growth performance and probably in disease resistance.

d. Existence/status/availability of branded products

Spray-dried plasma products for use in animal feeds were first developed and manufactured by APC, Inc. in the United States, and that company remains an important force in the market in the US and around the world with branded products including AP-920, Appetein and Solutein. A number of other manufacturers are now in the spray-dried plasma market. According to Russell (2001), the members of the North American Spray Dried Blood and Plasma Protein Producers Association are the following. Some of these companies may produce other blood products and not spray-dried plasma.

- APC, Inc.
- California Spray Dry
- DuCoa (now Trouw Nutrition)
- Harimax Inc.
- Hemotech
- Innovative Proteins
- Merrick's, Inc.
- Proliant
- Senimal, Inc.

e. Existing patents

There appear to be no patents on spray-dried plasma itself, but further processing required for certain products may be patented. For example, APC, Inc. holds a patent of a granulation process that makes the product less dusty and easier to handle in a feed mill.

f. Other attributes/considerations

For some time, the European Union banned the use of all blood products in animal feeds because of concerns about their possible role in spreading transmissible spongiform encephalopathies. That ban has now been eliminated and spray-dried plasma is again being used in pig feeds in Europe.

Dried Porcine Solubles

a. Description of products

Pig small intestinal mucosa is hydrolyzed for heparin extraction. The resulting hydrolysate is dried and can be added to young pig diets.

b. Proposed/demonstrated mechanism of action

We are not aware of a clearly defined proposed mechanism of action of dried porcine solubles. The product contains high concentrations of peptides. Short peptides can be absorbed from the digestive tract, and have been suggested to exert a variety of beneficial physiological effects (Moughan, 2001).

c. Efficacy

We reviewed refereed publications from three laboratories that reported the results of experiments designed to test the value of dried porcine solubles in the diet of young weaned pigs. In 8 of the 9 comparisons identified in these papers, growth rate was faster when the diet contained porcine solubles, with the unweighted mean increase in growth rate an astonishing 36% (Table 4). These limited empirical data certainly support inclusion of dried porcine solubles in a list of functional animal proteins.

However, some caution in this conclusion is urged for two reasons. First, a potential mechanism through which the product might provide benefits has not been identified. Second, the limited number of refereed reports makes this summary unusually susceptible to the positive bias resulting from failure to publish negative data, as described in the introduction. On the other hand, we acknowledge the existence of a sizable body of favorable data that have not been subject to peer review, which can be found at the primary supplier's website (http://www.nfprotein.com/).

d. Existence/status/availability of branded products

The dominant producer of dried porcine solubles products is a company called Nutra-Flo Protein and BioTech Products. The brand name is DPS 50RD.

e. Existing patents

The drying of the hydrolysate and use in animal feeds are not patented.

f. Other attributes/considerations

Early research on dried porcine solubles suggested improved growth after withdrawal of the product, but subsequent studies have not confirmed that proposed benefit.

Milk Proteins

a. Description of products

The most commonly used source of milk proteins in diets of young pigs is dried whey, a by-product of cheese manufacture. Whey is the liquid material remaining after the casein has clotted to form cheese. The high-quality whey products usually used in pig diets are dried gently to preserve functionality of the

immunoglobulins and other whey proteins. A number of other whey products are available for use in pig diets, including delactosed whey, demineralized whey, whey protein product and whey permeate.

Dry skim milk (nonfat dry milk) is milk with the fat and water removed, so it contains both the casein and whey proteins. It is used in young pig diets in some parts of the world, but not usually in the U.S. because of its price. There are also related products such as filled milk (dry skim milk with fat from a non-milk source added) available for use in pig diets.

Milk carries immunity and physiological signals from the mother to offspring, so it contains functional proteins.

Lactose (milk sugar) appears important in the diet of young pigs, but is outside the scope of this review.

b. Proposed/demonstrated mechanism of action

It is useful to divide milk proteins into two categories. Casein, the major protein in cow's milk, forms a curd in the presence of acid or certain enzymes. That curd formation occurs in the stomach of the young nursing animal, a process important in regulating the rate of flow of digesta into the intestine until the digestive system matures. Curd formation also occurs in the manufacture of cheese. All other milk proteins are grouped into the category of whey proteins, including immunoglobulins and many other physiologically active proteins (Pettigrew, 1975).

The immunoregulatory properties of milk derivates have been reviewed extensively elsewhere (Gill et al., 2000; Kussendrager and van Hooijdonk, 2000; Shah, 2000; Steijns and van Hooijdonk, 2000). Casein appears to affect the immune system as well as the flow of digesta through the digestive tract. Peptide-rich hydrolysates of alpha-casein have shown immune regulatory properties, with activity dependant on the action of digestive enzymes (Gill et al., 2000). Digestion of kappa-casein with either pepsin or chymotrypsin or digestion of alpha-casein with both pancreatin and trypsin significantly inhibited the proliferative responses of splenic lymphocytes and Peyer's patch cells (Otani and Hata, 1995). Both the carbohydrate and the polypeptide portions generated by enzymatic digestion are essential for inhibitory effects on proliferative responses of mouse spleen cells (Hata et al., 1998; Otani et al., 1995). In contrast, a casein polypeptide fraction, obtained from a pepsin-chymosin digestion, showed a mitogenic effect on primed lymph node cells and unprimed spleen cells of rats (Coste et al., 1992). Treatment of bovine alpha-casein with trypsin alone

results in the release of peptides that promote antibody formation and accelerate phagocytosis *in vitro* (Gill et al., 2000). Additionally, these latter peptides exhibit opioid-like properties *in vitro* and *in vivo*, which modulate immune function by enhancing lymphocyte proliferative responses, natural killer cell activity and leukocyte migration (Gill et al., 2000; Shah, 2000).

The whey fraction of milk contains immunoglobulins, high concentrations of active biopeptides with immune modulator properties, tissue growth factors and hormones (Hogarth et al., 2004; Low et al., 2003). The whey proteins lactoferrin, lactoperoxidase and lysozyme have antimicrobial properties (Shah, 2000). Some of these biopeptides may be present in milk products used in pig feeds.

Lactoferrin is predominantly found in the products of the exocrine glands located in the digestive, respiratory and reproductive systems, and is part of the innate defense against invading pathogens. This bioactive compound is secreted by the mammary gland, providing protection to the neonate (Steijns and van Hooijdonk, 2000). It is a dominant whey protein in human milk and plays an important role in iron uptake in the intestine (Shah, 2000). Lactoferrin exhibits both bacteriostatic and bacteriocidal activity against a range of microorganisms. It has been found to inhibit the growth of *E. coli*, *Salmonella typhimurium*, *Shigella dysenteriae*, *Listeria monocytogenes*, *Bacillus stearothermophilus* and *Bacillus subtilis*. The antimicrobial effect is mainly on the organisms that require iron as lactoferrin chelates iron, thereby depriving the organisms of a source of this nutrient (Shah, 2000; Steijns and van Hooijdonk, 2000). Lactoferrin also causes release of LPS molecules from the outer membrane of Gram-negative bacteria and acts directly as an antibiotic.

Lactoperoxidase is a member of the peroxidase family, a group of natural enzymes, widely distributed in nature. The peroxidases in the mammary, salivary and lachrymal glands are chemically and immunologically similar. The biological significance of lactoperoxidase is its involvement in the natural host defense system against pathogenic

microorganisms. In bovine milk it is a non-specific antimicrobial agent (Kussendrager and van Hooijdonk, 2000). Lactoperoxidase in the presence of H_2O_2 catalyses the oxidation of thiocyanate (SCN-) and produces an intermediate product with antimicrobial properties (Kussendrager and van Hooijdonk, 2000; Shah, 2000). Lactoperoxidase preparations have been used for controlling bacterial proliferation in biological products, food industry and cosmetics. The reaction products generated by the catalytic action of the lactoperoxidase system are harmless to mammalian cells (Kussendrager and van Hooijdonk, 2000).

Lysozyme is an antimicrobial enzyme which hydrolyses beta-1→4 linkages between N-acetylmuramic acid and 2-acetylamino-2-deoxy-D-glucose residues in bacterial cell walls, resulting in cell lysis. Milk lysozyme is active against a number of Gram-positive and some Gram-negative bacteria. There seems to be a synergistic action of lysozyme and lactoferrin against *E. coli* as the latter damages the outer membrane of Gram-negative bacteria and the organism becomes susceptible to lysozyme. Combinations of lysozyme and lactoferrin are more bacteriostatic than either of the proteins alone (Shah, 2000)

In addition to the antimicrobial activity described above, whey biopeptides modulate the action of the immune system. Several studies, both *in vitro* and *in vivo*, have shown that whey products inhibit the immune system. Consistent results from different laboratories have suggested that whey protein concentrate in the diet promotes immune tolerance to antigens present in diets, a desired phenomenon that prevents food allergy, autoimmune diseases, and acute and chronic inflammation (Enomoto et al., 1993; Fritsche et al., 1997; Pecquet et al., 1999). Whey protein concentrate suppressed T and B lymphocyte proliferative responses to mitogens in a dose-dependent fashion in vitro (Cross and Gill, 1999). Wong et al. (1997) demonstrated inhibitory effects of fractionated bovine whey proteins on cellular immune responses. In their study, both lactoferrin and lactoperoxidase were found to inhibit proliferation and interferon-gamma (IFN-γ) production of ruminant blood lymphocytes in response to mitogenic stimulation.

Interestingly, whey protein concentrate has also prompted up-regulation of the immune system. It increased gut mucosal antibody responses to orally-administered antigens in mice (Low et al., 2001). There was no effect on blood lymphocyte expression or splenic T or B cell mitogenesis, suggesting that the immunomodulatory action was not due to systemic lymphocyte activation. Similar results were reported from a related study (Low et al., 2003) in which mice fed whey protein concentrate produced elevated levels of antigen-specific intestinal and serum antibodies against all tested antigens, compared to mice that were fed a standard chow diet. Bovine whey fractions fed to mice stimulated higher blood total white cell, CD4+ and CD8+ lymphocyte counts and lowered the output of fecal oocysts in experimentally infected mice (Ford et al., 2001).

Other beneficial effects beyond antimicrobial action and immune regulation have been attributed to dietary supplementation of whey protein concentrate. Whey protein concentrate feed preparations enhanced intestinal calcium absorption in acute fashion (Zhao et al., 2005). However, the calcium absorption enhancing effect disappeared with chronic feeding in growing rats. Even when calcium absorption was only temporarily affected by 1% whey protein concentrate in the diet, an increase in whole body bone mineral content was observed. Similar enhancement of mineral absorption has been reported elsewhere (Bennett et al., 2000; Pantako and Amiot, 2001; Van Dael et al., 2005). Additionally, antiviral activity (Kanamaru et al., 1999; Kvistgaard et al., 2004), intestinal mucosa protective factors (Howarth et al., 1996; Tran et al., 2003) and inhibition of intestinal pathogens (DeLoach et al., 1990; Ingham et al., 2000; Manas et al., 2001) have been described for biopeptides contained in whey protein concentrate preparations.

As described above, multiple biological activities have been described for milk proteins. Immune regulation and antibacterial properties seem to be the most common attributes of milk derivates. The level of protection or benefit may depend on the quality of

the milk fraction included in animal feed. Quality of the whey product, level of inclusion, and feed preparation can influence the beneficial response described for these products.

c. Efficacy

Milk products are widely used in diets for newly weaned pigs, in spite of their greater cost than many other ingredients. They provide lactose, an energy source easily digested by the just-weaned pig that improves performance of young pigs when included in their diet (Mahan, 1992). They also provide a variety of milk proteins. These proteins are easily digested by the immature digestive system of the young pig, and there are reasons to speculate that they may also provide other benefits.

An early series of experiments (Pettigrew and Harmon, 1977; Pettigrew et al., 1977a; Pettigrew et al., 1977b) showed that artificially reared piglets can thrive when fed liquid diets made from dried skim milk. However, liquid diets devoid of intact milk proteins, based on egg whites plus lactose (Pettigrew and Harmon, 1977) or a tryptic digest of dry skim milk (Pettigrew et al., 1977b), were much less successful, with dramatically reduced growth rate and drastically increased diarrhea. Experiments with other species showed no large differences in nutritional value between diets based on dry skim milk and the other ingredients. The authors suggested that the intact milk proteins provided physiologically active components, mentioning the examples of casein and immunoglobulins.

Evaluation of the efficacy of milk proteins is especially challenging for two reasons. First, most milk products contain both proteins and lactose, and it is difficult to separate the potential benefits of those two components. We have ensured that the diets compared in our summary have similar levels of total lactose, but are not certain that the efficacy of the various lactose sources is equal. Second, there has historically been considerable variation in the quality of milk products, presumably due to drying processes and other manufacturing variables. A further complication is that several of the experimental diets used in tests of the value of milk proteins contained other milk products (dried whey or dried skim milk) in both the control and treatment diets, and that may diminish the need for the products tested.

As noted above different milk products have different physiological functions, so in our summary (Table 5) we have separated the studies by the milk product evaluated: casein, lactalbumin (a major whey protein) and total whey proteins (in dried whey or a whey protein product).

The response to casein in this dataset is disappointing, with 8 of 9 comparisons showing a detrimental effect. We have no way to judge whether this surprising result may be related to the quality of casein product used in these experiments. Perhaps the 4-week-old pigs used in these experiments were beyond the stage of physiological development at which the control of digesta flow obtained from the casein curd in the stomach is important.

We found only three tests of the effect of lactalbumin, and collectively they are not instructive. The primary physiological action generally attributed to lactalbumin is in lactose synthesis in the mammary gland, not in the nursing offspring, so perhaps a functional role in the diet should not be expected.

The responses to whey proteins are more encouraging, with increased growth rate in 9 of 11 tests. The unweighted mean increase in growth rate is 4.5%, a modest but useful response. Therefore, while the high digestibility of whey proteins may have influenced the results to some degree, the available data indicate that whey proteins provide some physiological benefits in addition to supplying bioavailable nutrients. It is not clear whether the benefits are due to immunoglobulins or some other component of whey.

In summary, some proteins in whey appear to provide specific benefits, but there is no evidence that casein does and the empirical evidence does not support a conclusion about benefits of lactalbumin.

d. Existence/status/availability of branded products

Milk protein products are manufactured and distributed by many milk processing companies, and they will not be listed here.

e. Existing patents

We are not aware of pertinent patents on applicable milk protein products or manufacturing processes or use of those products in animal feeds.

f. Other attributes/considerations

Milk proteins are especially susceptible to damage by overheating, especially when the product also contains lactose. That was a serious concern when milk products were first used extensively in diets for young pigs, but it now appears that most milk products on the market are manufactured under conditions that produce high-quality products.

Generic Egg Products

a. Description of products

Eggs that cannot be used for human consumption can be dried (often gently by spray-drying) and used in young pig diets. This procedure recovers valuable nutrients. It also recovers functional proteins, as immunity and physiological signals are transmitted from mother to offspring via the egg.

b. Proposed/demonstrated mechanism of action

The supposed beneficial effect of dietary egg product supplementation has been mainly attributed to antibodies. Since 1893, it has been recognized that avian maternal antibodies are transferred from serum to egg yolk to confer passive immunity to embryos and neonates. Chicken IgY antibodies can be compared to IgG antibodies obtained by conventional immunization methods (Tini et al., 2002). Chicken egg-yolk immunoglobulins do not interfere with mammalian IgG and they do not activate mammalian complement. These characteristics confer advantages to the application of IgY as antibiotic-alternative therapy (Tini et al., 2002).Thus, dietary supplementation of egg products may be expected to provide protection to neonate animals.

c. Efficacy

There exist surprisingly few reports in the literature of tests of the performance effects of conventional (non-immune) egg products (Table 6). Most of the experiments summarized have compared egg products to spray-dried plasma; the results show clearly that egg products are inferior to plasma as they reduced growth rate in all 7 relevant comparisons. This observation does not show that egg products lack useful physiological effects or cannot improve growth performance. It shows only that egg products are less effective than spray-dried plasma in increasing whole-animal growth.

On the other hand, two comparisons suggest that egg proteins are superior to soybean meal. We consider these data encouraging but inadequate to draw conclusions that egg proteins provide specific benefits unrelated to nutrient supply.

d. Existence/status/availability of branded products

Egg products for use in animal feeds are available in the market, often from companies that supply alternative feed ingredients. The egg products used in the experiments cited in Table 11 that were conducted in North America were manufactured by Inovatech Inc. and California Spray Dry Company.

e. Existing patents

We are not aware of pertinent patents on applicable egg products or manufacturing processes or use of those products in animal feeds.

f. Other attributes/considerations

If not manufactured properly, egg products may contain unacceptable levels of microbial contamination. *Salmonellae* are a specific concern.

Immune Egg Products

a. Description of products

Hens can be immunized against pig pathogens, so they produce antibodies against those pathogens and deposit them in the yolk of the eggs they produce. Feeding the yolk to pigs provides passive immunity to the target disease. A battery of hens may be immunized against several pig pathogens (one pathogen per hen) and the yolks combined to produce a multivalent passive immunity.

b. Proposed/demonstrated mechanism of action

As noted in the section on generic egg products, the supposed beneficial effect of dietary egg product supplementation has been mainly attributed to antibodies. Available research publications support the hypothesis that dietary supplementation of egg products helps protect animals against enteric disease. Diarrhea in neonatal and post-weaning pigs is a significant and persistent problem in pig production. Among the major

causes of diarrhea in piglets are enterotoxigenic *E. coli* (ETEC) strains expressing K88, K99 and 987P fimbrial antigens. Among these, ETEC K88+ antigen is the most prevalent form of *E. coli* infection of pigs worldwide (Jin et al., 1998).

Vaccination of laying hens with *E. coli* antigens provides an excellent and inexpensive source of antibodies targeting bacterial antigens. According to O'Farrelly et al. (1992), vaccinated hen eggs can contain as much as 200 mg of antibodies, almost exclusively in the yolk. Eggs from these vaccinated hens are collected after a high level of specific antibodies is reached in the egg yolk (Jin et al., 1998). *In vitro* studies demonstrated that chicken egg yolk antibodies to ETEC fimbrial antigens (K88 or F18ab) prevent *E. coli* with the target fimbria from binding to receptors in mucus isolated from the intestine of piglets (Imberechts et al., 1997; Jin et al., 1998).

Purified antibodies are enzymatically inactivated in the gastrointestinal tract of older pigs (Yokoyama et al., 1992). However, egg yolk antibodies pass the small intestine intact if fed as whole egg or mixed with egg white (Wiedemann et al., 1990) and can exert practical effects *in vivo*. In several studies, dietary chicken egg yolk significantly reduced adherence and colonization of ETEC strains in neonatal pigs (Imberechts et al., 1997; Marquardt et al., 1999; Owusu-Asiedu et al., 2002; Owusu-Asiedu et al., 2003a; Yokoyama et al., 1997; Yokoyama et al., 1992; Zuniga et al., 1997). Egg yolks from hens immunized with enterotoxigenic *E. coli*, when fed to rabbits for 4 days, protected these animals from developing diarrhea when challenged with the same enterotoxigenic organism. Additionally, antibodies precipitated from the immune egg yolks are equally protective, suggesting that the antibodies rather than some other component of the egg yolk was providing the protection (O'Farrelly et al., 1992).

Taken together, these results indicate that immune egg products may be suitable immunotherapeutic and immunoprophylactic agents for reducing gastrointestinal disorders in piglets (Imberechts et al., 1997; Marquardt et al., 1999; Owusu-Asiedu et al., 2002; Owusu-Asiedu et al., 2003a; Yokoyama et al., 1997; Yokoyama et al., 1992; Zuniga et al., 1997). In conclusion, beneficial effects of dietary supplementation of egg products can be attributed to antibacterial antibodies and subsequent reduction of gastrointestinal bacterial infections.

c. Efficacy

Several studies have shown immune egg products to be powerful in protecting young pigs from diarrhea caused by *E. coli* (Table 7). These products reduced the incidence and (or) severity of diarrhea in 23 of 31 cases, with most responses being dramatic. The immune egg products have been shown effective against *E. coli* with four important fimbrial types, including K88 and F18.

Spray-dried porcine plasma containing a high antibody titer against specific *E. coli* fimbrial antigens was shown to be remarkably effective in protecting pigs against diarrhea caused by *E. coli* with those fimbria, and there was no further improvement from specific immune egg products (Owusu-Asiedu et al., 2002; Owusu-Asiedu et al., 2003a). When the protection provided by the spray-dried porcine plasma was eliminated by (1) autoclaving the plasma product , (2) replacing it with a spray-dried animal plasma without detectable antibody activity against the specific *E. coli* fimbria , or (3) replacing it with pea protein isolate (Owusu-Asiedu et al., 2003a), the immune egg product provided protection.

Some studies (Chernysheva et al., 2004; Chernysheva et al., 2003; Zuniga et al., 1997) found a lack of protection from immune egg products. It appears the principal immune egg product used in the experiments of Chernysheva et al. (2003, 2004) was the same as that used by (Owusu-Asiedu et al., 2002; Owusu-Asiedu et al., 2003a), who found it to be protective. The reasons for the differences in response among laboratories are unknown. It was not reported whether the diets in the experiments of Chernysheva et al. (2003, 2004) contained spray-dried porcine plasma. The failure to find benefits in some cases serves as a reminder that while immune egg products appear to be very useful tools, they may not yield the desired results in every situation.

The experiments summarized in Table 7 studied a wide range of immune egg products, and it is difficult to compare their potency. We suggest the antibody titer may be the most useful comparison of the amount of product used across experiments, but the definition of titer varies across laboratories and is not always explicitly reported. Where the immune egg products were mixed into feeds, the calculated titer of the complete feed is reported in Table 7. A low titer may explain the lack of protection afforded in some situations.

In all of the challenge experiments cited in Table 7, the immune egg products were introduced either before or at the same time as the challenge organism. Evidence was not found that immune egg products can

cure an existing enteric infection. In fact, there is evidence that pretreating pathogenic *E. coli* with an immune egg product prevents binding of the organism to intestinal mucosal cells, but that the product does not dislodge organisms already bound to intestinal cells (Jin et al., 1998), casting doubt on the therapeutic value of these products.

We have not found tests of these products in pigs against other enteric pathogens. Immune egg products have been shown to offer protection against salmonella and rotavirus in mice and calves (Kuroki et al., 1993; Kuroki et al., 1994; Yokoyama et al., 1997; Yokoyama et al., 1992).

In summary, the empirical data provide confidence that immune egg products can be very effective in enhancing disease resistance in young pigs.

d. Existence/status/availability of branded products

Several companies market branded immune egg products, but fewer companies produce them. Among the companies producing such products are the following:

- Camas Inc. (U.S.)
- NutraTech Inc. (Canada)
- Trouw Nutrition International (U.S.)
- Zymefast (Canada)

The only commercial product used in the studies cited in Tables 13-14 was obtained from Nutratech Inc.

e. Existing patents

There are existing or pending patents related to design of the immunogen or other aspects of immunization methods used to produce the immune egg products.

f. Other attributes/considerations

The regulatory status of immune egg products may evolve as these products become more widely used. They are likely to be listed on labels as dried egg products, because if they are claimed to provide protection against disease, they will be considered drugs subject to intensive testing before they can be marketed.

Immune egg products may be unstable during pelleting.

Summary

Either appropriately produced immune egg products or spray-dried plasma can provide protection against enteric infections in young pigs when included in the diet. Spray-dried plasma also increases growth rate dramatically. While some of the increased weight gain of pigs consuming plasma may be lost after withdrawal of the plasma, much of the advantage appears to be retained. The limited data available indicate that dried porcine solubles also increases growth rate dramatically, but conclusions must be tentative because of the small body of data. Whey proteins increase growth rate modestly, but there is no persuasive evidence that either casein or lactalbumin does so. There is insufficient data to support conclusions on whether conventional egg products may be beneficial.

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