

# **Influence of Dietary Plasma Proteins on Supporting Animal Immunity Systems**

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## **Introduction**

Spray-dried plasma (SDP) or spray-dried serum (SDS) are ingredients that are collected and processed to preserve the functional characteristics of the proteins. Spray-dried plasma or serum is a diverse mixture of functional components consisting of immunoglobulins, albumin, fibrinogen, lipids, growth factors, biologically active peptides (defensins, transferrin), enzymes and other factors that have biological activity within the intestine independent of their nutritional value. Spray-dried plasma is primarily used as an ingredient blended into dry feed or milk replacers. Spray-dried serum is an ingredient used in colostrum supplements and/or other liquid feeding applications.

Spray-dried plasma is used extensively in nursery pig feed to enhance feed intake, growth, and feed efficiency during the post-weaning period. The beneficial effects of SDP are more pronounced under production conditions with high pathogen exposure than with low pathogen exposure. Numerous studies involving challenge with pathogenic bacteria, viruses or protozoa have demonstrated reduced mortality and morbidity with feeding spray-dried animal (bovine or porcine) plasma to various animal species (swine, calves, poultry, shrimp).

Several modes of action of SDP have been proposed. Collectively, these proposed actions suggest that oral consumption of SDP may conserve immune response resources through interactive mechanisms between the intestine and other immune system tissues. The purpose of this review is to focus on SDP modulation of the animal's immune system and how it may be utilized in economically important applications of animal production.

## **Mechanisms of Spray-Dried Plasma**

Literature reviews (Coffey and Cromwell, 2001; van Dijk et al., 2001) indicate an average improvement in body weight gain, feed intake, and feed efficiency of 25, 21, and 4%, respectively due to consumption of SDP by weanling pigs. The magnitude of growth and feed intake response to SDP is difficult to explain from a purely nutritional effect. Ermer et al. (1994) reported that both palatability and feed intake were improved when pigs were fed diets containing SDP compared to dried skim milk suggesting that

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SDP improved feed intake and growth simply because it was more palatable. However, Jiang et al. (2000ab) reported that feeding SDP to pigs, pair-fed to the same intake as control pigs, improved efficiency of dietary protein utilization by decreasing intestinal amino acid catabolism and reducing plasma urea N concentration. Furthermore, the researchers noted that SDP reduced cellularity of the lamina propria of the small intestine suggesting reduced local inflammation.

The beneficial effects of SDP are more pronounced under production conditions with high pathogen exposure than with low pathogen exposure (Stahly et al., 1994; Coffey and Cromwell, 1995). Likewise, similar observations have been reported in broilers (Campbell et al., 2003) and turkeys (Campbell et al., 2004a). Numerous studies (Table 1) involving challenge with pathogenic bacteria (*E. coli*, *Salmonella*, *Pasteurella multocida*), viruses (rotavirus, coronavirus, white spot syndrome virus) or protozoa (*Cryptosporidium parvum*) have demonstrated reduced mortality and morbidity when feeding spray-dried animal (bovine or porcine) plasma to various animal species (swine, calves, poultry, shrimp).

More recent evidence supports the concept that oral consumption of SDP reduces or modulates the over-stimulation of the inflammatory response. Touchette et al. (2002) reported reduced cytokine mRNA expression (TNF- $\alpha$ , IL-1 $\beta$ , and IL-6) in multiple tissues (hypothalamus, pituitary, adrenal, spleen, thymus, and liver) of pigs orally consuming SDP and challenged with lipopolysaccharide (LPS). Bosi et al. (2004) reported that feeding SDP to pigs challenged with enterotoxigenic *E. coli* K88 reduced inflammation as indicated by improved growth, reduced salivary IgA secretion, decreased intestinal mucosal damage and reduced pro-inflammatory cytokine expression in the gut. They concluded that SDP protects against *E. coli* K88 infection by maintaining mucosal integrity, enhancing specific antibody defense and decreasing inflammation in the intestine. Nofrarías et al. (2006) reported that feeding SDP to non-challenged weaned pigs reduced inflammation as indicated by reduction in intraepithelial lymphocytes and lamina propria cell density in the large intestine. They concluded that SDP lowers activation of the immune system of non-challenged pigs similar to challenged pigs in experiments from Bosi et al. (2004) and Touchette et al. (2002).

The mechanisms suggested to elicit these results are that SDP reduces attachment, adhesion, and replication of the organism (antigen-antibody interactions), facilitates tissue repair, or reduces the overall inflammatory response both systemically or locally. Collectively, the mechanism of action of dietary SDP on growth performance involves modulation or management of the immune system and the degree of immune activation may limit the availability of energy for growth, alter structural integrity of the intestinal barrier, and limit other productive functions (ie. growth, reproduction, and pregnancy).

## **Influence of SDP on Intestinal Inflammation and Gut Barrier Maintenance**

Inflammation of the intestine can cause multiple effects such as edema, leukocyte infiltration, vasodilatation, reduced nutrient absorption, increased epithelial permeability due to altered barrier function, and immune system activation. To further elicit the effects of SDP on specific immune response of the intestine, Pérez-Bosque et al. (2004) developed a rat model for evaluating impact of SDP during intestinal inflammation. The rats were challenged with *Staphylococcus aureus* enterotoxin B (SEB). Staphylococcal enterotoxins (superantigens) are potent activators of the immune system. These superantigens activate a high percentage of T cells by cross-linking major histocompatibility complex (MHC) class II molecules with a moiety on the variable portion of the  $\beta$  chain of the T-cell receptor (TCR) (McKay, 2001). Feed intake and growth rate was monitored in the challenge model. Although challenged, feed intake and growth rates were unaffected, indicating mild inflammation.

Pérez-Bosque et al. (2004) reported less fecal water content, reduced  $\gamma\delta$ -T lymphocytes, and reduced percentage of cytotoxic cell populations in organized gut associated lymphoid tissue (GALT) populations (ie. Peyer's patches) of rats fed SDP and challenged with SEB indicating reduced intestinal immune activation when supplemented with SDP. Using the same experimental model, Garriga et al. (2005) reported that SDP reduced the effects of SEB on glucose transport in the rat intestine as determined by increased sodium glucose transporter 1 (SGLT1) expression in the villous apex. Glucose absorption was 8-9% greater in rats fed SDP when challenged with SEB indicating improved nutrient absorption when animals were fed SDP.

Intestinal permeability was evaluated by Pérez-Bosque et al. (2006) using the same SEB challenge model. Rats administered SEB had increased intestinal permeability as assessed by both structural and functional measurements. Structurally, SEB administration in rats resulted in a reduction of epithelial tightness as observed by reduced expression of ZO-1 (in tight junction) and  $\beta$ -catenin (an adherent junction). Functionally, both dextran (4 kDalton) and horse radish peroxidase (HRP, 40 kDalton) were used to assess flux across the intestinal wall as tracers of mucosal permeability to toxins and food antigens. Treatment of SEB increased HRP and dextran intestinal flux suggesting higher luminal permeability to macromolecules during intestinal inflammation. Dietary supplementation of SDP reduced the effects of SEB by reducing dextran and HRP paracellular flux across the intestinal epithelium. Thus, SEB-induced intestinal inflammation as indicated by increased permeability across the intestinal mucosa and lower expression of tight junction proteins (ZO-1 and  $\beta$ -catenin), can be reduced by SDP supplementation. They concluded that SDP supplementation can in part reduce alterations of epithelium structure, thus improving intestinal mucosal barrier function.

Intestinal inflammation can be mediated by cytokines or other pro-inflammatory mediators. Pérez-Bosque et al. (2007) evaluated the effect of SDP supplementation on cytokine expression in rats challenged with SEB. Administration of SEB increased all pro-inflammatory cytokines (IL-6, IFN- $\gamma$ , and TNF- $\alpha$ ) in both organized (Peyer's patches)

and diffuse (lymphocytes and mucosal lamina propria) GALT. The increase in cytokines can explain the reductions in intestinal barrier function observed in previous experiments (Pérez-Bosque et al., 2006). Spray-dried plasma supplementation reduced the increase in pro-inflammatory cytokines induced by SEB. The effects of SDP on cytokine expression can explain previous observations of reduced mucosal immune activation, as well as, effects on nutrient transport and intestinal permeability. Moreover, SDP supplementation increased IL-10 (anti-inflammatory cytokine) secretion. Thus, SDP supplementation reduced SEB-induced increase in pro-inflammatory cytokines with the effects potentially mediated by enhanced IL-10 secretion.

The immune system in response to stress and antigen exposure stimulate pro-inflammatory cytokines in the brain which reduces motivation to eat (Kent et al., 1996) and interacts with growth hormone and insulin-like growth factor (IGF-1) to suppress cell growth (Kelly, 2004). The more recent evidence that SDP reduces the over-stimulation of pro-inflammatory cytokines strongly suggests that this is a primary mechanism of action of SDP in restoring feed intake of animals and reducing the deleterious effects of disease and other stressors. Inflammatory events occur throughout the life-cycle of animals and use of SDP to modulate the inflammatory response in applications beyond the weaning period is now being explored.

### **Spray-Dried Plasma and Productive Functions of Calves**

Immune activation due to various stressors (ie. disease challenge, co-mingling, heat stress, weaning, etc.) can affect economically important production functions such as growth, lean tissue deposition, reproduction, and lactation. Depending upon the degree of immune activation and/or stress, animals may experience reduced growth (Johnson, 1997; Spurlock 1997), reduced milk production (O'Brian et al, 2007), or pregnancy loss (Erlebacher et al., 2004). Maintenance of intestinal barrier function may partially reduce activation of the immune system; thereby, reducing losses associated with various stressors.

The use of SDP to reduce the effects of enteric challenges has been evaluated by several researchers in calves. Quigley and Drew (2000) challenged 36 colostrum-deprived Holstein bull calves with *E. coli* K99 at 3 d of age. Calves were fed commercial calf milk replacers containing no additive, an antibiotic (neomycin and oxytetracycline) or SDP at 3.3% of the formula. All calves showed signs of enteric infection following oral challenge; however, calves fed either antibiotic or SDP had lower mortality and morbidity (number of days with diarrhea) than calves fed the control milk replacer. Based on attitude score, calves consuming SDP or antibiotic were more active and vigorous.

Hunt et al. (2002) orally challenged 24 calves with  $10^8$  oocysts of *Cryptosporidium parvum* at 8 d of age. Calves were fed either soy protein concentrate or SDS in a milk replacer. Oral challenge caused significant fecal shedding of *C. parvum* oocysts, diarrhea, increased intestinal permeability, reduced villous surface area and reduced intestinal lactase activity. Calves consuming SDS had a 33% reduction in oocyst

shedding, 33% reduction in peak diarrheal volume, 30% reduction in total intestinal permeability, 15% increase in villous surface area, and more rapid recovery following challenge. They concluded that SDS reduced the effects of *C. parvum* by reducing the number of viable parasites, facilitating intestinal repair, and reducing attachment and replication of the infection.

Arthington et al. (2002) fed 12 Holstein bull calves milk replacer containing 0 or 160 g/d of an additive containing SDS as a therapy following oral challenge with bovine coronavirus on d 0. Feeding the additive containing SDS improved average packed cell volume, respiration rate, and feed intake compared to calves fed diets without the additive containing SDS. The authors concluded that supplementation of milk replacer with the additive containing SDS improved rate of recovery in calves following coronavirus challenge.

Quigley et al. (2002) reported the effects of feeding SDP or an additive containing bovine SDS, fructooligosaccharides and minerals/vitamins in two studies utilizing 240 Holstein bull calves purchased from sale barns and dairy farms. Calves were usually within one week of age and in various stages of failure of passive transfer. In experiment 1, calves fed an additive containing bovine SDS tended to have fewer days with diarrhea, lower use of electrolytes, and improved BW gain from d 29 to 56. Addition of SDP to milk replacer did not influence any parameter measured. In experiment 2, calves fed additive containing bovine SDS or milk replacer containing SDP had lower mortality (4.4 vs. 20%) and tended to have improved fecal scores and fewer days with scours. Antibiotic use was lower when calves were fed the SDS additive. Indices of enteric health (incidence of scours and treatment with antibiotics and electrolytes) were improved when SDP was added to milk replacer throughout the milk feeding period or as a serum additive during the first 15 d of the milk feeding period, when calves were most susceptible to enteric pathogens. The primary difference between experiments 1 and 2 was the overall level of stress. Calves used in experiment 1 were purchased from more dairy farms than sale barns and the experiment was conducted at an optimal time of the year (i.e., weather closest to the thermoneutral zone), calf milk replacer (CMR) contained all milk protein, and there was a general lack of enteric challenge. Conversely, Experiment 2 was conducted during a cold period of the year, the calves were fed CMR containing soy protein and clinical symptoms related to enteric and respiratory pathogens occurred during the trial. Generally, these data suggest that calves fed SDP – whether as SDP in the CMR or as a serum additive – will respond to SDP, particularly when the overall level of challenge is significant.

Quigley et al. (2003) also reported about bovine or porcine derived SDP added to calf milk replacer. The milk replacers were formulated to contain whey protein concentrate (WPC) as the primary protein source or WPC plus 5% spray-dried bovine or porcine plasma. Intake, change in body weight, feed efficiency, morbidity, and mortality were determined. Mortality was 25, 7.5, and 5% in calves fed WPC, spray-dried-bovine or porcine plasma treatments, respectively. Morbidity, measured as the number of days that calves had diarrhea was reduced by about 30% when spray-dried

bovine or porcine plasma were fed. Calves had diarrhea for 6.4, 3.9, and 4.7 d during the 42-d study when fed milk replacer containing WPC, spray-dried bovine or porcine plasma, respectively. Fecal scores tended to be reduced and feed efficiency tended to be improved when spray-dried bovine or porcine plasma were fed. Mean body weight gains from d 0 to 42 were 231, 261, and 218 g/d for calves fed WPC, spray-dried bovine or porcine plasma, respectively. Overall, inclusion of spray-dried bovine or porcine plasma in milk replacer reduced morbidity and mortality of milk-fed dairy calves.

Heat stress is another type of environmental stress which disrupts intestinal barrier function resulting in gut leakage and increased serum endotoxin (Lambert, 2004). When intestinal barrier function is compromised, the immune system is stimulated resulting in reduced intestinal function (i.e. nutrient absorption), which ultimately impacts productive functions. Recently, a field trial was conducted during heat stress conditions using calves at a commercial ranch in the western US. Mortality rates historically increased during these months and the increase in mortality was attributed to heat stress. An additive containing SDS supplemented to calves in the milk replacer reduced mortality compared to previous year mortality rates (4.3 vs. 2.0%). The personnel at the site noted that calves fed the additive containing SDS were more vigorous and ready to drink their second feeding during the heat of the afternoon.

In summary, the use of SDP is well accepted in animal agriculture. Spray-dried plasma and/or spray-dried serum reduce the over-stimulation of the immune response in animals (ie. pigs, rats), thereby conserving nutrient utilization for supporting the immune response and allowing nutrients to be utilized for productive purposes. Similar modulation effects of SDP or SDS on inflammation and intestinal barrier function may be occurring in other animals such as calves as well. Research continues to elucidate the important role of these proteins in SDP in animal agriculture.

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**Table 1. Summary of results from experimental challenges using SDP.**

Specie	Pathogen	Results	Author	Year
Pigs	<i>E. coli</i>	↓ fecal score	Borg et al.	1999
Pigs	Salmonella	↓ fecal score	Borg et al.	1999
Pigs	<i>E. coli</i>	↑ ADG, ↓ IgA	Bosi et al.	2004
Pigs	<i>E. coli</i>	↑ ADG, ↑ <i>Lactobacilli</i>	Torrallardona et al	2003
Pigs	<i>E. coli</i>	↑ ADG	Campbell et al.	2001
Pigs	<i>E. coli</i>	↓ shedding	Deprez et al.	1996
Pigs	Rotavirus	↓ diarrhea	Corl et al.	2007
Pigs	<i>E. coli</i>	↓ fecal score	Nollet et al.	1999a
Pigs	LPS	↓ cytokine mRNA expression	Touchette et al.	2002
Pigs	<i>E. coli</i>	↑ ADG, ↓ fecal score	Van Dijk et al.	2002
Calves	Coronavirus	↑ recovery	Arthington et al.	2002
Calves	<i>Crypto. Parvum</i>	↓ scours, ↓ shedding	Hunt et al.	2002
Calves	<i>E. coli</i>	↑ survival, ↑ ADG, ↓ scours	Nollet et al.	1999b
Calves	<i>E. coli</i>	↑ survival, ↑ ADG, ↓ scours	Quigley and Drew	2000
Shrimp	White Spot Syn.Virus	↑ survival, ↑ ADG	Russell & Campbell	2000
Poults	Pasteurella	↑ survival, ↑ ADG	Campbell et al.	2004b

# **SESSION NOTES**