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Natural and artificial hyperimmune solutions: Impact on health in puppies

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Contents

Colostrum and milk are complex mammary secretions providing the puppy with many nutritional and immunological factors, which play a crucial role for its correct development and survival. In the case of colostrum and/or milk intake deficiency, puppies are at increased risk of infectious diseases. This work reviews the various nutritional hyperimmune supplementations proposed to provide a passive immune protection and to positively impact puppies' health. Some strategies rely on canine immunoglobulins: canine colostrum banking and canine serum/plasma supplementation. Others involve heterologous sources of antibodies and other immune factors: bovine colostrum or hyperimmune egg powder. Among the different solutions evaluated from birth to weaning, canine plasma and hyperimmune egg powder showed promising beneficial effect on puppies' health. Canine plasma seems to positively impact not only growth (increased growth during the neonatal period), but also digestive health (higher species richness of intestinal microbiota) and the general health (tendency of lower morbidity). Puppies supplemented with hyperimmune egg powder presented increased neonatal growth and decreased risk of canine parvovirus infection. Nevertheless, natural canine maternal colostrum and milk ingestion remains the optimal guarantee for puppies' health and survival, as a source of immunity, energy and growth factors.

1 | INTRODUCTION

The survival of canine newborns, as that of other altricial species, depends entirely on maternal care. At birth, puppies are unable not only to walk, hear or see, but also to ingest solid food, regulate their body temperature, and even urinate and defecate spontaneously (Grundy, 2006). Moreover, their immune status is immature and of weak efficacy (Day, 2007). This makes puppies highly susceptible to infections not only during the neonatal period (i.e. the first 3 weeks of life), but even until weaning (i.e. until 7–9 weeks of life).

1.1 | Global immune protection

Due to endotheliochorial placentation in dogs (Stoffel, Friess, & Hartmann, 2000), only 1%–7% of total immunoglobulin G (IgG) concentration obtained by passive immune transfer are from the transplacental origin (Bouchard et al., 1992; Poffenbarger, Olson, Chandler, Seim, & Varman, 1991). The vast majority of IgG are thus absorbed after birth, thanks to the colostrum intake over the first hours of life. Due to the immaturity of the intestinal mucosa over the first hours following birth (Paulsen, Buddington, & Buddington, 2003; Schwarz & Heird, 1994), macromolecules can be absorbed from the gut lumen into the newborn's bloodstream. However, progressive intestinal barrier closure, consequence of the differentiation of the digestive mucosa, decreases the absorption by the gut of macromolecules (including IgG) (Chastant-Maillard et al., 2012). Absorption rate of immunoglobulins (i.e. per cent of ingested colostrum immunoglobulin G transferred to the bloodstream), limited at birth to 40%, drops to 14% at 8 hr and is null after 24 hr.

Puppies with blood IgG concentrations below 230 mg/dl at 2 days are determined to suffer from a deficit of passive immune transfer. Their risk of dying during the neonatal period is ninefold higher than in

puppies with correct transfer (blood IgG concentration >230 mg/dl at 2 days of life; Mila, Feugier, et al., 2014).

The absence of notable transplacental antibodies transfer and the total dependence on colostrum intake make puppies highly susceptible to infections, with septicaemia being the most frequent cause of death over the neonatal period (41%–65% of pre-weaning losses). The most frequent symptoms are lethargy, loss of sucking reflex, crying, diarrhoea, weight loss and hypothermia. Puppies usually die within hours after showing symptoms or suddenly with no preceding clinical signs (Münnich & Küchenmeister, 2014; Meloni et al., 2014; Nielen et al., 1998). Bacteria most often isolated from affected newborns are *E. coli*, *Streptococci* and *Staphylococci* (Meloni et al., 2014; Münnich & Küchenmeister, 2014). In total, the mortality and morbidity rates in dogs over the pre-weaning period are high, reaching approximately 20% and 35% of puppies, respectively (Konde, Gitau, Kiptoon, & Gakuya, 2015; Mila & Chastant-Maillard, 2014). Large breed puppies (expected adult body weight >25 kg) seem to be especially sensitive, with a higher percentage of sick puppies over the first week of life than for small breeds (<25 kg): 55% (71/128) versus 31% (64/206), respectively (unpublished data).

As appropriate passive immune transfer decreases the risk of infectious diseases and neonatal losses (Mila, Feugier, et al., 2014), dog breeders should be encouraged to assist suckling in puppies over the first hours after birth. Yet, in some circumstances, either from puppy or maternal origin, colostrum intake is limited or absent (e.g. weak puppy, large litters, agalactia, lack of maternal behaviour, death of the mother), precluding adequate passive immune transfer. Alternatives to colostrum are then required. The role of colostrum in energy provision can be efficiently ensured by milk replacers; however, the most critical aspect is to find colostrum substitution for efficient passive immune transfer to the newborns.

1.2 | Local immune protection

The immune function of mammary secretions does not terminate with the intestinal barrier closure. While IgG is the dominant Ig class of mammary secretions on the first day of lactation, percentage of IgA increases very rapidly, becoming the main Ig type from Day 7 to the end of lactation (Schäfer-Somi, Bär-Schadler, & Aurich, 2005). Unlike IgG supporting systemic immunity after being absorbed into the bloodstream, IgA has a local role in the mucosal digestive immunity. Pathogen-specific antibodies obtained via milk intake are crucial for the defence of the newborn, virus neutralization, inhibition of gut colonization and agglutination of microorganisms within the gastrointestinal tract (Hurley & Theil, 2011). The role of milk antibodies is supported by observations of puppies naturally exposed to canine parvovirus type 2 (CPV2): despite non-protective serum CPV2-specific antibody titres (hemagglutination inhibition titre <1:80) at 2 days of age, none of thirteen puppies got infected before 6 weeks of life (Mila, Grellet, et al., 2014). Those results suggest that not only the global, but also local immunity provided by milk plays a role in the protection of newborn dogs. Therefore, administration of a milk substitute, supplemented with specific immunoglobulins, could be considered in at-risk animals, such as hand-reared puppies or puppies from large litters.

The objective of this review was to describe different hyperimmune solutions aiming to decrease a risk of infections and health issues during the pre-weaning and early post-weaning period in dog, either through systemic passive immune transfer or by local immunity.

2 | COLOSTRUM BANKING

In cattle and horses, colostrum banking is routinely performed by breeders. Feeding of neonates with frozen/thawed colostrum could also be performed in breeding kennels. The colostrum collected from bitches living in the same kennel is the optimal solution to replace maternal colostrum in newborn puppies. It brings not only antibodies directed against local pathogens, but also large amounts of energy. In order to collect colostrum of high immune quality, bitches should be milked after cleaning of the skin, by a gentle teat massage approximately 24 hr after whelping. This delay is a compromise between the acquisition of passive immunity by the litter of the donor bitch and the kinetics of colostrum IgG concentration. Indeed, colostrum IgG concentration decreases by more than 50% over the first 24 hr after parturition (3830 mg/dl at 4 hr after the expulsion of the first puppy versus 1730 mg/dl at 24 hr (Albaret, Mila, Grellet, & Chastant-Maillard, 2016)). Subsequently, IgG concentration continues to decrease, reaching approximately 200 mg/dl as early as Day 7 and remaining low until the end of lactation (Schäfer-Somi et al., 2005). One- to five-ml samples should be stored in polypropylene or glass tubes and immediately frozen at −20°C. Before administration, frozen colostrum should be warmed up to 30–35°C, for example with a baby bottle warmer; microwaves should be avoided (due to immunoglobulin destruction). Freezing/thawing only slightly affects the immune properties of the colostrum and milk (Lawrence, 1999). A minimal volume of 1.5 ml/100 g of body weight (bw) should be administrated *per os* to puppies within the first 8 hr of life. This dose administered within that time frame was found to ensure IgG concentration above the minimal protective level (Chastant-Maillard et al., 2012).

3 | BOVINE COLOSTRUM

Bovine colostrum is produced in large quantities and is easily available. Lyophilization does not alter its immune quality (Klobasa, Goel, & Werhahn, 1998). However, early administration of bovine colostrum for acquisition of systemic immunity has never been evaluated in the newborn dog. It is questionable as heterologous colostrum would not provide antibodies specific for canine pathogens (CPV2 or canine coronavirus CCoV for example, highly pathogenic for puppies). Hyperimmune bovine colostrum and milk (with antibodies against human rotavirus) have been used with success against gastrointestinal infections in humans (Hurley & Theil, 2011), but never in the dog.

Nevertheless, immune factors provided by colostrum and acting on the infant's health are not limited to immunoglobulins (Table 1). As demonstrated in early weaned piglets, bovine colostrum

supplementation could ameliorate the digestive health via decrease of gastric pH, increase of *Lactobacilli* sp. concentration or improvement in intestinal mucosa development (Huguet & Le Dividich, 2011). Bovine colostrum was tested as a source of local immunity in puppies at weaning. When administered to puppies from 50 to 60 days of age (0.5 g of bovine colostrum powder/day/miniature breed puppy), an improvement in faecal consistency in supplemented puppies was stated by the authors, although the graph presented in their paper does not support this result (Giffard, Seino, Markwell, & Bektash, 2004).

4 | CANINE SERUM/PLASMA

Immune therapy with intravenous immunoglobulin administration is routinely used in immunocompromised human patients (Hemming, 2001; Orange et al., 2006). Therapeutic, but also prophylactic intravenous administration of immunoglobulins in preterm babies (with a deficit of transplacental immune transfer) decreases the risk of neonatal sepsis and death (Jenson & Pollock, 1997). As intravenous injection in the newborn dog is difficult and risky to perform, subcutaneous and oral routes have been used (Table 2). In studies on colostrum-deprived puppies, none of the groups supplemented with canine serum (orally or subcutaneously) obtained IgG concentrations comparable with mean levels acquired after spontaneous maternal colostrum intake (Bouchard et al., 1992; Poffenbarger et al., 1991). When canine plasma was used orally as an additional source of immunoglobulins in free-suckling puppies, and not as a colostrum replacer in colostrum-deprived puppies, serum IgG concentration was not increased (Mila, Feugier, et al., 2014). After subcutaneous injection (2–4 ml canine serum/100 g bw administered at birth), the minimal protective IgG concentration (>230 mg/dl) was achieved in one study (mean IgG concentration in puppies' blood: 264 and 446 mg/dl, respectively; Bouchard et al., 1992). However, according to the authors, large amounts of liquid accumulated at the site of injection (a total of 16 ml), with gross lesions revealed at necropsy. Serum subcutaneous injection indeed may provoke severe skin necrosis.

Despite limited interest on serum IgG concentration after birth, early oral plasma administration displays other benefits on puppies' health (Mila et al., 2015). Plasma was administrated orally (1.5 ml/100 g bw via a feeding tube) twice within the first 8 hr of life (before intestinal barrier closure) to 28 puppies. Since Day 2 of life,

TABLE 1 Immunological components in the colostrum and milk (adapted from Tizard, 2001 and Hamosh, 2001)

Component	Systemic immune action	Local immune action
Immunoglobulins: IgG IgA IgM	Source of antibodies providing systemic specific immunity	Source of antibodies providing local specific immunity
Immune cells: Macrophages Neutrophiles Lymphocytes	Active defense Enhance passive immunity Immunoregulatory and immunostimulatory	Active defense
Lactoferrin		Bacteriostatic Antiviral action Immunomodulation Anti-inflammatory
Cytokines		Immunomodulation Maturation of the immune system
Lysozyme		Bacterial lysis Immunomodulation Binding to bacterial lipopolysaccharides
Lactoperoxidase		Anti-inflammatory action
Oligosaccharide		Prevent bacterial binding to epithelial surfaces
Glycoproteins: Mucin Lactadherin		Prevent bacterial binding to epithelial surfaces
Nucleotides	Immunomodulation	Promote growth of *Lactobacillus bifidus* Immunomodulation
Hormones: Prolactin Cortisol Thyroxine Insulin Growth factors		Improve differentiation of intestinal lymphoid tissue Promote development of intestinal host-defense mechanism

TABLE 2 Effect of canine serum/plasma administration at birth on puppies' blood IgG concentration at 24 h (Bouchard et al., 1992) or 48 hr of life (Mila, Feugier, et al., 2014; Poffenbarger et al., 1991)

	Study concerned		
	Bouchard et al. (1992)	**Poffenbarger et al. (1991)**	**Mila, Feugier, et al., (2014)**
Puppies included	37	25	149
Separation from the dam	Yes	Yes	No
Supplement used	Canine serum	Canine serum	Canine plasma
Time of administration (after birth)	At birth and 12 hr	At birth	At 4 and 8 hr
Total volume administered (per 100 g bw)	4 ml	2.2 ml	3 ml
Serum/plasma IgG concentration	1820 mg/dl	2100 mg/dl	1430 mg/dl
Total IgG administered (per 100 g bw)	73 mg	46 mg	43 mg
Blood IgG concentration in puppies after			
Colostrum intake (controls[a])	3366 mg/dl	1697 mg/dl	701 mg/dl
Serum/plasma oral administration	199 mg/dl	149 mg/dl	690 mg/dl
Serum/plasma SC administration	264 mg/dl[b]	214 mg/dl	NA

bw, body weight; SC, subcutaneously; NA, not applicable.

[a]Unlimited colostrum intake.

[b]Serum SC injection with 2 ml/100 g bw only at birth.

supplemented puppies received the plasma at the same dose every 2 days until weaning (i.e. 56 days), aiming to provide the immune supplement locally. Blood donors and puppies receiving canine plasma were housed within the same breeding kennel. No supplementation was provided to the control group (*n* = 30).

A significant effect of the plasma administration on weight gain during the neonatal period was observed in large breed puppies. Over the first 3 weeks of life, supplemented large breed puppies gained almost 600 g more than the controls (mean ± SD; 1408 ± 217 g vs. 815 ± 376 g; *p* < .001; Figure 1).

Plasma supplementation was also associated with an increased microbial diversity and altered microbial communities in the gut (Mila et al., 2015). The number of observed species (species richness) was significantly higher in supplemented versus control puppies as early as 2 days of life, and it remained stable until weaning (Figure 2). Neonatal gut colonization impacts its development: intestinal microbiota not only enhances the anatomic development of intestinal epithelium, but also stimulates the maturation of intestinal lymphoid tissue, thereby improving the immune response (Arrieta, Stiemsma, Amenyogbe, Brown, & Finlay, 2014).

Moreover, the microbial communities were significantly different between the supplemented and control group at Day 2, Day 42 and Day 56 (*p* = .003; *p* = .003; *p* = .04, respectively). *Clostridium* sp. and *Peptostreptococcaceae* (responsible for neonatal septicaemia) were less represented in supplemented puppies at Day 2, whereas *Lactobacillus* sp. (presenting immunomodulating activity) was more represented in the supplemented group at Day 42.

The effect of plasma supplementation on morbidity during the neonatal period was also investigated (authors unpublished data). Puppies were examined twice weekly from birth to 1 month of age. Presence of nasal and ocular discharges, diarrhoea, dehydration or
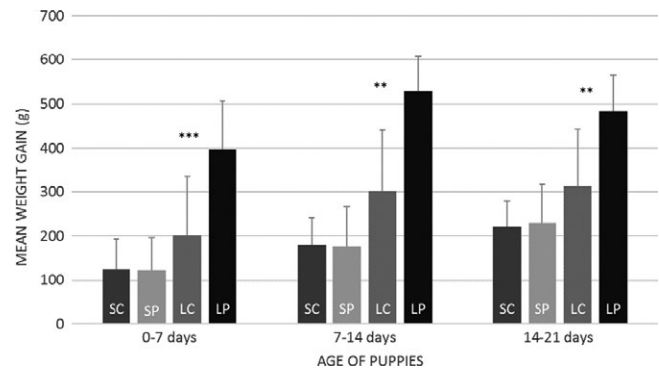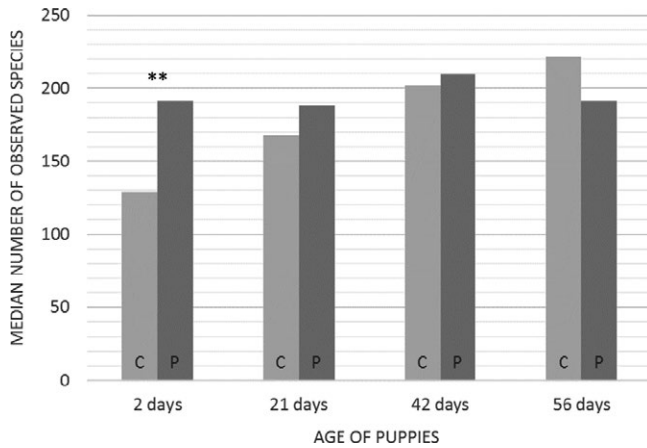

FIGURE 1 Effect of canine plasma supplementation on puppies' weight gain during the neonatal period (mean ± SD; 58 puppies; SC—control small breed puppies *n* = 22; SP—plasma supplemented small breed puppies *n* = 15; LC—control large breed puppies *n* = 8; LP—plasma supplemented large breed puppies *n* = 13). Asterisks indicate a significant difference between control and supplemented puppies within each breed size and over one week of life using linear mixed model with litter as a random term (****p* < .001; ** *p* < .01)

other clinical signs was recorded. Among 70 supplemented puppies, 14 (20%) presented at least once a sign of a disease over the study period versus 27 out of 79 control puppies (34%). Thus, puppies receiving canine plasma tended to be at lower risk of a disease than control puppies (*p* = .07).

To summarize, oral plasma supplementation (twice within the first 8 hr of life and since Day 2 of life, every 2 days until 56 days), allowed greater weight gain, increased intestinal microbiota diversity and tended to reduce risk of disease in puppies. Canine serum/plasma administration since birth to weaning, although not directly improving global blood IgG levels and not providing puppy with energy, seems to have a beneficial impact on puppies' health.

4.1 | Hyperimmune egg powder

Another source of heterologous immunoglobulins is hyperimmune egg powder. Antibodies specific for canine pathogens are transferred to


FIGURE 2 Effect of canine plasma supplementation on puppies' digestive microbiota. Number of observed species within the intestinal microbiota (median; 58 puppies; C—control puppies n = 30; P—plasma supplemented puppies n = 28). Asterisks indicate a significant difference between control and supplemented puppies at the time of faecal sample collection analysed using observed species diversity index (**p < .01)

the egg yolk in hens vaccinated against canine microbes (Van Nguyen, Umeda, Yokoyama, Tohya, & Kodama, 2006). Harvested and dried egg yolk can be easily stored and used as an oral supplement. Oral passive immunization with egg powder was proposed as an alternative to antibiotics in farm animals. A meta-analysis based on 61 experimental protocols evidenced that hyperimmune egg powder administration significantly reduces the risk of gastrointestinal infections in mice, poultry, piglets and calves (Diraviyam et al., 2014).

The impact of hyperimmunized egg powder administered to newborn puppies before intestinal barrier closure was evaluated on neonatal health (Mila, Grellet, Mariani, Feugier, & Chastant-Maillard, 2016). Hyperimmunized egg powder was obtained from hens vaccinated against CPV2 and *E. coli*. Among 334 puppies included in the study, 169 were allocated into the supplemented and 165 into the control group. Supplemented puppies received hyperimmunized egg powder diluted in milk replacer (250 mg in 1.5 ml of restored milk [Babydog Milk, Royal Canin, Aimargues, France]/100 g bw) once within the first 8 hr of life via a feeding tube. Milk replacer only was given at the same dose and delivered by the same method to the control group. Puppies remained non-separated from their dams during the study period and they could suckle their dam freely.

Supplemented large breed puppies presented a significantly greater weight gain over the entire neonatal period compared to the controls (824 ± 349 g vs. 662 ± 334 g; p = .03; Figure 3).
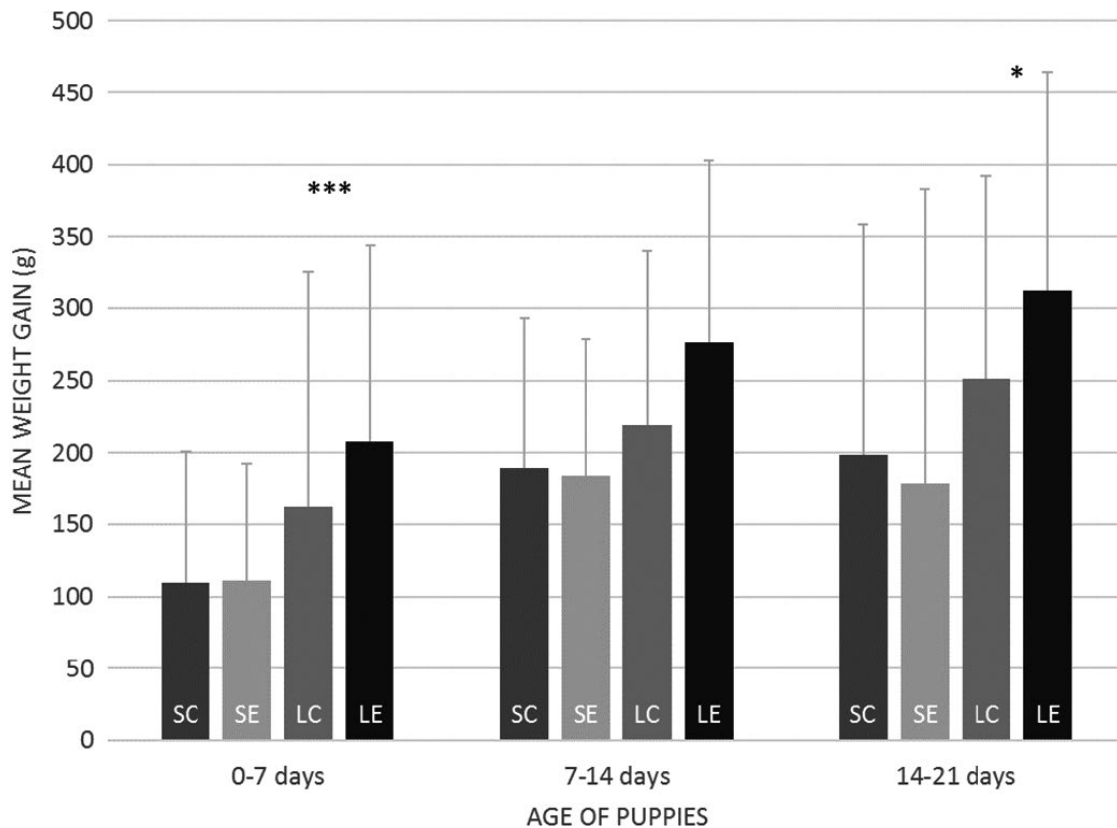
FIGURE 3 Effect of hyperimmune egg powder supplementation on puppies' weight gain during the neonatal period (mean ± SD; 334 puppies; SC—control small breed puppies n = 102; SE—egg supplemented small breed puppies n = 104; LC—control large breed puppies n = 63; LE—egg supplemented large breed puppies n = 65). Asterisks indicate a significant difference between control and supplemented puppies within each breed size and over 1 week of life using linear mixed model with litter as a random term (***p < .001; * p < .05)

The beneficial effect of hyperimmune egg powder was demonstrated also when administered daily as a diet supplementation in early weaned puppies. Alaskan husky puppies (n = 36) receiving egg powder from hens immunized against several bacterial species (*E. coli*, *Salmonella* spp.) tended to have improved faecal quality and increased faecal IgA (Reynolds & Knorr, 2006). With an egg powder containing CPV2-specific antibodies, Van Nguyen et al. (2006) evidenced a protective effect of supplementation after an experimental CPV2-challenge: puppies receiving 2 g of egg powder three times per day (n = 3) presented no symptoms of the disease, whereas control puppies (n = 4) showed weight loss, vomiting and diarrhoea. Caution is required, however, in regard to the low sample size.

Hyperimmune egg powder, administered before or after gut closure, thus appears as a promising prophylactic and therapeutic tool targeted against specific canine pathogens.

5 | CONCLUSIONS

The perfect hyperimmune solution, approaching colostrum or milk properties, should provide the newborn not only with specific passive immunity, but also nutrients and energy. Canine serum/plasma shows beneficial effect on health of the newborn dog, but is poorly caloric. Bovine colostrum, although providing energy, does not contain canine pathogen-specific antibodies. Hyperimmune egg powder, bringing energy and targeted against canine pathogens, seems to be to date the most promising strategy to improve puppy's health.

CONFLICT OF INTEREST

HM, AG, BG, JS, JS and SCM have no conflict of interest to declare. CM and AF are Royal Canin employees.

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