

# Clinical applications of pheromones in dogs

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*Abstract:* In dogs, six major sources of pheromones exist: the facial area, the pedal complex, the perianal complex, the genital complex and the mammary complex. In the prevention and treatment of canine behavioral disorders the synthetic analogue of dog appeasine, the Dog Appeasing Pheromone (DAP®) is frequently utilized. Many studies have demonstrated the utility of DAP® in the treatment of the separation related problems, sound sensitivity, adaptation to a new environment, transport-related behavior problems and in the protection of dog welfare in the shelter. Some criticisms have been made about method and procedures used, recognized as not appropriate to provide evidence of the effectiveness of DAP®. These criticisms were later countered but it is clear that the study of these particular substances requires careful methodological rigor, as many interfering factors may be present. Since DAP® is not systemically absorbed, there is no toxicity or side effects which allows for its safe use alone or combination with psychotropic drugs.

*Key Words:* dog appeasing pheromone; separation related problems, sound sensitivity.

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## Introduction

The term pheromones was coined in 1959 by Peter Karlson and Martin Luscher, who defined them as “substances which are secreted to the outside by an individual and received by a second individual of the same species, in which they release a specific reaction, for example a definite behavior or a developmental process” to define a substance secreted by an individual and received by a second subject of the same species in which it induces a behavioral or physiological change (Karlson & Lüscher, 1959).

In dogs, six major sources of pheromones exist (Pageat & Gaultier, 2003): the facial area, the pedal complex, the perianal complex, the genital complex and the mammary complex.

The area of the cheek and perioral glands brings together a whole set of secreting structures spread throughout the chin, lips, vibrissae, cheeks and ceruminous glands of the ear duct and of the external ear.

The pedal complex consists of the pedal glands of the four legs, structures present both in the plantar pads and in the skin of the interdigital region.

The perianal complex is formed by the supracaudal glands, the circumanal glands and the anal sacs. The genital complex includes sebaceous glands of the prepuce or the vulva and urethral or genital mucous glands together.

The mammary complex has been discovered in recent years. The first pheromone isolated in this area was in the sow and shortly thereafter the same kind of pheromones in bitches, mares, cows, ewes, queens and does was isolated. These pheromones, called appeasines for their appeasing action, are secreted by the sebaceous glands of the sulcus between the two mammary chains. The ap-

peasines of the bitch have the same chemical structure as those of the other species; three fatty acids could be considered as the “mammal appeasing message”: oleic acid, palmitic acid and linoleic acid, always associated in the same ratio. The other components could be considered as the species-specific message, which, in the bitch, are in the following order: myristic acid, lauric acid, pentadecanoic acid and stearic acid. The secretion appears 3 to 4 days after parturition and persists 2 to 5 days after the weaning of the puppies (about 4 months of age).

In the prevention and treatment of canine behavioral disorders the synthetic analogue of dog appeasine, the Dog Appeasing Pheromone (DAP®) is frequently utilized. Numerous researches have been carried out but some criticisms have been made about method and procedures used, recognized as not appropriate to provide evidence of the effectiveness of DAP® (Frank et al., 2010). These criticisms were later countered (Pageat et al., 2010) but it is clear that the study of pheromones requires careful methodological rigor, as many interfering factors may be present.

### DAP® and separation related problems

Among the behavioral disorders, the separation related problems are the ones that probably derive more benefit from the use of the pheromone. These disorders are quite common and account for approximately 40% of cases submitted to behavioral counseling. Pheromones have been proven effective in the treatment of separation related problems compared with Clomipramine: in a 2005 study (Gaultier et al., 2005), fifty-seven dogs that showed signs of distress when separated from their owners (destructiveness, excessive vocalization and house soiling) and hyperattachment were used in a randomized, blind trial to assess the potential value of DAP® in reducing those behaviors. For ethical reasons, there was no placebo group and the effects of the pheromone were compared with the effects of Clomipramine. The undesirable behaviors decreased in both groups but the overall assessment by the owners indicated that there was no significant difference between the two treatments, although there were fewer undesirable events in the dogs treated with DAP® and the administration of the pheromone appeared to be more convenient (Tab. 1).

Tab 1. Percentages of dogs reporting specific behavioral signs after 28 days treatment with DAP or Clomipramine (\*significant difference  $p < 0.05$ ).

<b>Behavioral Signs</b>	<b>Clomipramine Dogs %</b>	<b>DAP Dogs %</b>
Destruction	70	73
Vocalization	67	67
Defecation/urination	67	40*
Sleeping problems	22	23
Excessive licking	37	43
Feeding problems	33	57
Hypersalivating/vomiting	22	13
Lack of adaption to change	41	30

Another critical situation in which the dog can manifest separation problems is the hospitalization; even in this case the use of pheromones can have positive effects as shown by a research of Kim et al. (2010). The study assessed the effect of DAP® on 10 typical separation-related behavioral signs in hospitalized dogs. A DAP® treated group was compared with a placebo control group. There was overall amelioration of the signs, without ‘vigilance’ and ‘anorexia’ in the DAP-treated dogs; marked decreases were noted in elimination ( $p = 0.038$ ), excessive licking ( $p = 0.005$ ), and pacing ( $p = 0.017$ ).

Hospitalization is often associated with the perioperative stress response, a physiologic reaction to surgery and various associated conditions (e.g. pain, analgesia and anesthesia-induced dysphoria, human handling and confinement to a hospital cage) that may be perceived as threatening by an animal. DAP<sup>®</sup> appears to affect behavioral and neuroendocrine perioperative stress responses by modification of lactotropic axis activity. A study (Siracusa et al., 2010) was performed on 46 dogs housed in animal shelters and undergoing elective orchiectomy or ovariectomy. Intensive care unit cages were sprayed with DAP<sup>®</sup> solution or sham treated, with the carrier used in the solution, 20 minutes prior to use. Dogs (n = 24 and 22 in the DAP<sup>®</sup> and sham treatment exposure groups, respectively) were placed in treated cages for 30 minutes before and after surgery. Indicators of stress (i.e. alterations in behavioral, neuroendocrine, immune, and acute-phase responses) were evaluated perioperatively. Behavioral response variables, salivary cortisol concentration, WBC count, and serum concentrations of glucose, prolactin, haptoglobin, and C-reactive protein were analyzed. Behavioral response variables and serum prolactin concentration were influenced by DAP<sup>®</sup> exposure. Dogs exposed to DAP<sup>®</sup> were more likely to have alertness and visual exploration behaviors after surgery than dogs exposed to sham treatment. Decreases in serum prolactin concentrations in response to perioperative stress were significantly smaller in dogs exposed to DAP<sup>®</sup>, compared with findings in dogs exposed to the sham treatment. Variables examined to evaluate the hypothalamic-pituitary-adrenal axis, immune system, and acute-phase responses were unaffected by treatment.

DAP<sup>®</sup> can improve dog welfare also in the veterinary clinic as demonstrated by a research performed on 15 dogs (Mills et al., 2005). The behavior and emotional state of these dogs, known to be fearful of the veterinary clinic was evaluated during a standardized 5 min waiting room procedure and standardized 2 min consultation room procedure prior to a sham clinical examination, in the presence of DAP<sup>®</sup> and placebo. Subjects acted as their own controls and were semi-randomly allocated into treatment groups to control for order effects. A triple blinding procedure was used in order to remove bias from the assessment of video recordings of the dogs, with two independent raters used to analyze the video recordings of the behavior of dogs during the test procedures. The raters showed good, and similar, agreement in their evaluation of both the specific behavior of the dogs and their putative emotional state (relaxed, aroused and anxious). The results suggest that the use of DAP<sup>®</sup> in the clinic was associated with greater relaxation of the dogs but there was no effect on aggressive behavior during the clinical examination.

## DAP<sup>®</sup> and sound sensitivity

Some studies have shown the effectiveness of pheromones in the treatment of sensitivity to sound: many dogs are sensitive to loud noises such as fireworks or thunderstorms. Often the cases of sensitivity to noise are not referred, unless the symptomatology is not clear and the pathology does not entail big problems to the quality of life of the dog or of the owner. The most frequently reported symptoms are: tachypnea, tremors, restlessness and tendency to hide; more rarely episodes of destruction or inappropriate elimination are described. In a 2007 study (Levine et al., 2007) the efficacy of two self-help CD based desensitization and counter-conditioning programmes with the use of DAP<sup>®</sup>, the training progress and owner compliance were evaluated. Fifty-four dogs were recruited for an 8-week period of training and were separated into two treatment groups, each using a different CD based programme. After implementing the CD programme for the 8-week period without any personalized instruction, two telephone follow-up interviews were completed after periods during which fireworks are commonly used. Assessment of efficacy was measured using both owner reports of its natural response (i.e. the dog's behavior in the home) and video footage of behavior in response to a novel recording of the problem sound (i.e. the dog's behavior in the behavior clinic) pre- and post-treatment.

The majority of change with respect to the dogs' response to the CD occurred during the first month of training with no significant change during the second month of training. With respect to real exposures, there was a significant reported improvement at both follow-up interviews in both the total severity scores and the global fear scores. There was significant improvement in the mean severity score of all individual behaviors at the first follow-up with the exception of "vigilance" behavior. Inappropriate elimination was the only behavior to be completely resolved by the second follow-up. No difference was found in the video recordings of fear behaviors occurring in response to a novel CD recording pre-treatment versus post-treatment.

Sound-induced fear and anxiety can be ameliorated by using a DAP® collar. In a research of Landsberg et al. (2015), twenty-four beagle dogs, divided into two treatment groups (DAP® and placebo) balanced on their fear score in response to a thunderstorm recording, were exposed to two additional thunderstorm simulation tests on consecutive days. Dogs were video-assessed by a trained observer on a 6-point scale for active, passive and global fear and anxiety (combined). Both global and active fear and anxiety scores were significantly improved during and following thunder compared with placebo on both test days. DAP® significantly decreased global fear and anxiety across 'during' and 'post' thunder times when compared with baseline. There was no significant improvement in the placebo group from baseline on the test days (Landsberg et al., 2015).

### DAP® and new environment adaptation

Another situation potentially stressful for the dog is the adaptation to a new environment following the adoption, widely recognized as being stressful for a puppy, because it involves major changes. The puppy's maternal bond is broken and it is moved to a new social and physical environment with new rules (Elliot & Scott, 1961; Pettijohn et al., 1977; Serpell & Jagoe, 1995). The potential value of DAP® in reducing stress in puppies newly adopted from a pet shop was assessed in a research of Gautier et al. (2008). The trial was triple-blinded and placebo-controlled. After their arrival at the pet shop, 32 puppies were fitted with a DAP collar and 34 were fitted with a control collar, according to a randomisation protocol. Adopting owners were contacted by telephone, three and 15 days after they had adopted a puppy, to obtain information about the puppy's integration into the family, and particularly about any signs of distress shown by the puppy when it was socially isolated. All the isolated puppies from the control group vocalised during the first night. Signs of distress, particularly vocalization (Fig. 1), were significantly lower in the DAP® group on day 3 and throughout the rest of the study and vocalization during the night ceased significantly sooner in this group.

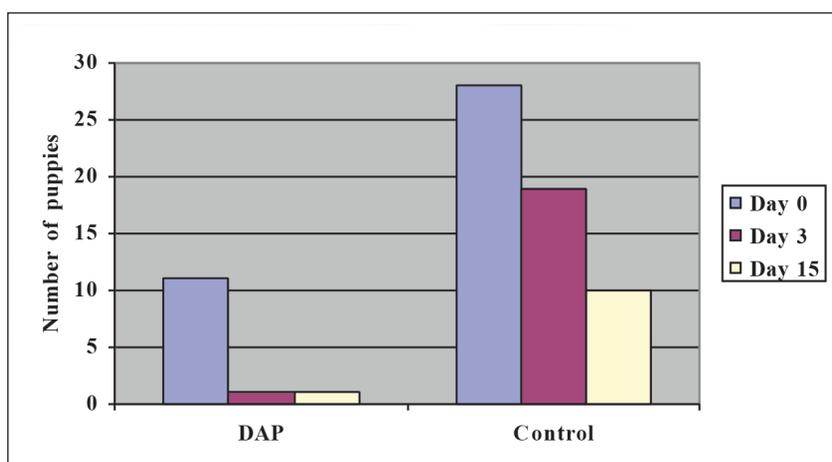


Fig. 1. Number of puppies showing vocalization in the control and DAP® group.

These differences were observed in puppies of small, medium and large breeds. The DAP® collars had no effect on the incidence of house soiling (Gaultier et al., 2008).

Adopting a puppy is successful only if its arrival enriches family life, but difficulties in coping with life in the new family setting may severely affect the development of the puppy's human-animal bond. Unexpected fear reactions towards new people and dogs will impair the puppy's social skills and may often compel owners to further restrict its social life. It therefore appears to be important to control any signs of fear displayed by puppies after their adoption. DAP® has been revealed useful for this purpose, as shown by a study realized in 2009 by Gaultier and colleagues. The study was triple-blinded, randomized and placebo-controlled. It used 66 puppies (32 fitted with a DAP® collar and 34 control) and the adoptive owners were contacted by phone three days and 15 days after they had adopted the puppy to question them about its reactions to specific situations eliciting fear. Fifteen days after the treatments significantly fewer of the puppies with the DAP® collars showed signs of fear when facing unfamiliar people at home and/or during outings. This difference was irrespective of breed size (Gautier et al., 2009).

Adaptation to a new socio-environment might represent a very hard step for sheltered dogs, because of a higher level of difficulty in coping with unfamiliar conditions. A study, performed by Osella and colleagues (Osella et al., 2015) investigated the effects of DAP® in dogs, adult and puppies, re-homed from rescue shelters. The study was designed as a prospective open-label clinical trial. Significant decreases were observed in adult dogs for wandering in the house restlessly ( $p=0.022$ ) and hiding fearfully in protected corners ( $p=0.033$ ), whereas in puppies treatment with DAP® significantly ( $p<0.05$ ) improved the reaction towards unfamiliar dogs ( $p=0.048$ ) and wandering in the house restlessly ( $p=0.022$ ). In both adults and puppies a significant improvement in interaction with owners was observed. In particular, "looking continuously for the owners" and "following the owners everywhere like a shadow" were significantly improved ( $p=0.0012$  and  $0.0016$  respectively) in adult dogs. Separation reactions revealed a significant decrease ( $p<0.05$ ) and in puppies the tendency to vocalize in absence of the owner was also significantly reduced ( $p=0.0029$ ). Both adults and puppies showed a decreased tendency to wake suddenly in the night ( $p=0.024$  and  $p=0.026$  respectively) and wander around the home ( $p=0.012$  and  $p=0.026$  respectively). In contrast, for house-training no significant difference was reported in adults, whereas for puppies there was a significant decrease ( $p<0.05$ ) in the mean scores for urination and/or defecation wherever in the house and after coming home. Data regarding the overall assessment suggested a significant improvement in all the efficacy variables considered in the study. The analysis of owners' degree of satisfaction at the final visit showed that DAP® treatment was considered successful by 84.4% of owners.

## DAP® and transport-related behavior problems

Transport-related behavior problems might be improved by spraying DAP® in the car, about 10 minutes prior to travel as shown by a double blinded study performed on 32 dogs. The effect of DAP® in comparison to a placebo spray over 5 trips of at least 15 minutes in length, was evaluated. There was a significantly improvement in the DAP® group. In the control group, no further improvement was seen after the second trip while the DAP® group improved throughout the 5 trips. There was a greater effect on physical signs (e.g. salivation, vomiting, urination and defecation) rather than behavioral signs (e.g. barking, motor activity) (Gaultier et al., 2003).

## DAP® in the shelter

DAP® might also be used to reduce stress of dogs in a shelter. Its behavioral effects were evaluated in adult dogs housed in a public animal shelter (Tod et al., 2005). Barking amplitude (dB) and

the frequency of discrete behavioral responses to two temperament tests associated with fear, separation and excitable behavior were recorded in 37 treatment and 17 control dogs. Mean barking amplitude and barking frequency were significantly reduced in dogs subject to DAP® exposure for 7 days ( $p < 0.001$  and  $p < 0.04$ , respectively), though peak values were not significantly altered. There was also some reduction in the barking amplitude of dogs during the 1 min recovery period, following a distraction. Following 7 days of DAP® exposure, there were significant differences in resting ( $p = 0.03$ ), barking ( $p < 0.04$ ) and sniffing frequency ( $p = 0.01$ ) (Fig. 2) in response to a friendly stranger. There were no highly significant differences in response to a neutral stranger.

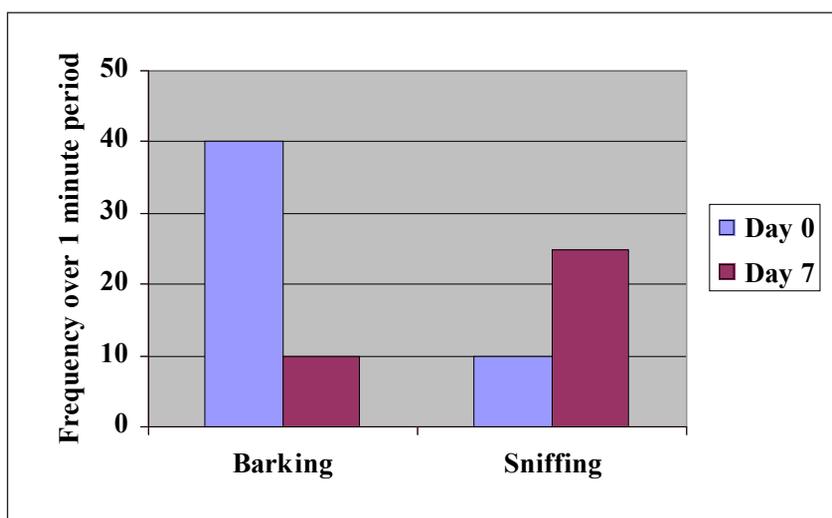


Fig. 2. Barking and sniffing frequency in dogs at day 0, before exposure to DAP®, and at day 7, following continuous exposure to DAP®, in response to friendly stranger test.

## Conclusion

Many studies confirm the utility of pheromones in the treatment and prevention of behavioral problems. Since they are not systemically absorbed, there is no toxicity or side effects, which allows for their safe use alone or combination with psychotropic drugs (Landsberg 2006).

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## Applicazioni cliniche dei feromoni nel cane

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### *Sintesi*

Nel corpo del cane esistono sei maggiori zone di produzione di feromoni: l'area facciale, il complesso podale, quello perianale, il complesso genitale e quello mammario.

Nella prevenzione e nel trattamento dei disordini comportamentali del cane   frequentemente utilizzato l'analogo sintetico del feromone di appagamento canino (DAP<sup>®</sup>) prodotto da particolari ghiandole situate nel solco intermammario.

Molti studi hanno dimostrato l'utilit  del DAP<sup>®</sup> nel trattamento dei problemi da separazione, della sensibilit  ai rumori, dell'adattamento ad un nuovo ambiente, dei problemi legati al trasporto e nella tutela del benessere in canile.

Alcune critiche sono state avanzate per quanto riguarda i metodi e le procedure utilizzate in alcune ricerche in quanto ritenute non appropriate a fornire evidenze scientifiche dell'efficacia del DAP<sup>®</sup>.

Queste critiche hanno in seguito avuto risposta da parte di altri ricercatori.   comunque evidente che lo studio di queste sostanze molto particolari richiede un estremo rigore metodologico poich  possono esservi molti fattori interferenti.

Dal momento che il DAP<sup>®</sup> non   assorbito per via sistemica, non esistono effetti tossici o collaterali e ci  permette un suo utilizzo sicuro, da solo o in associazione con farmaci psicotropi.