



Nutritional supplementation in cases of canine cognitive dysfunction—A clinical trial

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Abstract

Canine cognitive dysfunction (CCD) is a clinical condition, which impacts significantly on the lives of elderly dogs and their owners. It is hypothesised that nutritional supplementation can be used in the management of the condition and this trial was designed to investigate the therapeutic effects of a specific supplement when compared to a placebo. The trial was conducted in a clinical context and involved 20 UK veterinary practices, giving geographical spread across the country. The duration of the trial was 56 days, including a baseline period of 7 days and a post trial period of 7 days. There was a significant difference between the treated and the placebo groups in relation to improvement in their scores for disorientation, changes in interaction and house soiling behaviour at day 21, day 28 and day 42. These results support the clinical practice of nutritional supplementation as a valuable component of the therapeutic approach in cases of canine cognitive dysfunction.

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1. Introduction

Canine cognitive dysfunction (CCD) is a clinical condition, which is primarily identified by behavioural changes in the aged patient. The prevalence within the UK has not been accurately established, but a recent survey of 981 owners of dogs over 7 years of age (Vetplus Ltd., 2005 Survey of owners of geriatric pets) suggested that approximately one third of dogs showed signs

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of confusion, restlessness and less enjoyment of life, while one in five exhibited an increased incidence of house soiling problems. A study in the USA involving 97 spayed female and 83 castrated male dogs aged between 11 and 16 years aimed to determine the prevalence of age-related behavioural impairment in a randomly chosen population of dogs. The results of this study showed that 28% (22/80) of 11–12-year-old dogs demonstrated impairment in one or more categories of behavioural signs associated with CCD and 10% (8/80) had impairment in two or more behavioural categories. In contrast 68% (23/24) of 15–16-year-old dogs had impairment in one or more categories and 35% (12/34) had impairments in two or more categories (Neilson et al., 2001). CCD is a neurodegenerative disorder resulting in a decline in higher brain functions, including those involved in memory and learning. It is believed to resemble Alzheimer-type dementia in humans in both its symptomology and pathophysiology (Cummings et al., 1996). For example, the brains of 20 old dogs aged between 8 and 18 years of age were compared with the brains of 10 younger dogs and revealed pathological changes affecting the meninges, choroid plexi, meningeal and parenchymal vessels, neurons and glial cells. The authors noted the similarities between the age-related changes in canine and human brains and concluded that the dog could be used as a natural model for the study of ageing and neurodegenerative disease in humans (Borras et al., 1999). Similarities in underlying pathology have been reflected in a similar approach to treatment for the canine and human conditions of age related pathology and since, in human medicine, single ingredient supplements have proven successful in aiding symptoms that are normally associated with Alzheimer's disease it has been hypothesised that nutritional manipulation can also be used in the management of the canine condition. Research in the human field has indicated that increased vulnerability to oxidative stress is associated with the ageing process and that among the most effective agents that counteract the effects of that process are fruit derived polyphenolics with high antioxidant activity (Joseph et al., 2000). A range of other human studies have also supported the efficacy of nutritional manipulation as a treatment modality for cases of age related behaviour changes in humans (Prasad et al., 2000; Kontush and Schekatolina, 2004; Youdim et al., 2000). Similarly a variety of studies in the veterinary field have supported the proposal of nutritional manipulation, particularly with anti-oxidants as part of the treatment regime in cases of CCD and reported significant effects of modified diets in laboratory based studies (Milgram et al., 2001, 2002a,b, 2004). They also found that the improvement related to the test food was clearest in the most difficult cognitive tasks. However, in a clinical setting it is recognised that owners of older dogs are often reluctant to alter their pet's diet and that the presence of systemic disease may also limit the use of diets specifically designed for the control of CCD. The proposal of a nutritional supplement is therefore of clinical interest and the aim of this study was to assess the efficacy of such a product in a clinical field trial.

In this study the use of a nutritional supplement (Aktivait[®], VetPlus Ltd., Lytham St Anne's, UK), rather than an alternative diet, was investigated. Constituents of Aktivait[®] (see Table 1) include a range of antioxidants and free radical scavengers including *N*-acetyl cysteine, which is a primary precursor to glutathione (Pocernich et al., 2000), α -lipoic acid, Vitamins C and E (Zandi et al., 2004), L-carnitine and Co-enzyme Q10. Essential fatty acids docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) are also included, both of which have been shown to be beneficial in influencing the effects of brain ageing in humans (Horrocks and Yeo, 1999; Kyle et al., 1999; Otsuka, 2000). In addition Aktivait[®] contains phosphatidylserine, which is a natural phospholipid, the main physiological effect of which is to enhance and maintain the cell activities based on the functionality of the plasma membrane (Samson, 1987). Clinical studies have demonstrated the role of phosphatidylserine in cognitive function of human patients (Cenacchi

Table 1
Aktivait[®] composition

| | Small breed < 10 kg | Medium and large breed > 10 kg |
|--------------------|---------------------|--------------------------------|
| DHA/EPA | 35 mg | 70 mg |
| Vitamin C | 20 mg | 40 mg |
| N-Acetyl cysteine | 20 mg | 40 mg |
| L-Carnitine | 13.5 mg | 27 mg |
| α-Lipoic acid | 10 mg | 20 mg |
| Vitamin E | 10 mg | 20 mg |
| Acetyl-L-carnitine | 5 mg | 10 mg |
| CoQ10 | 1 mg | 2 mg |
| Phosphotidylserine | 1 mg | 2 mg |
| Selenium | 25 mcg | 50 mcg |

et al., 1993) and in experimental animals, administration of phosphatidylserine rapidly induced dose-dependent improvements during learning and memory tests (Suzuki et al., 2001).

2. Materials and methods

This trial was designed to investigate the therapeutic effects of a specific nutritional supplement when compared to placebo. Cases were recruited from a clinical population. The trial was conducted through 20 UK veterinary practices giving reasonable geographical spread across the country in order to compensate for any geographical variations in canine populations. One veterinary surgeon in each of the practices was responsible for recruiting cases for the trial and was supported by one veterinary nurse, who assisted with the completion of trial paperwork and with the conduct of telephone interviews where necessary. The veterinary surgeons that were approached to take part in the trial were general practitioners with an interest in behavioural medicine and were familiar with the diagnosis and treatment of CCD cases. The trial monitor visited each veterinary surgeon prior to commencement of the trial and gave training in relation to the trial design, the criteria for inclusion and exclusion and completion of the necessary paperwork. The study population was selected from the clinical population of the veterinary practices taking part. There were no selection criteria imposed in terms of breed, sex and neuter status. The diagnosis of canine cognitive dysfunction was based on clinical signs in the categories of disorientation, changes in social interaction, changes in sleep–wake cycle and alterations in previously conditioned behaviours. This diagnostic process is well established in the field of veterinary behavioural medicine and supported by a range of publications (Landsberg et al., 2003; Bain et al., 2001; Heath, 2002; Landsberg and Araujo, 2005). The ability to toilet in an appropriate location and on an acceptable substrate is established during the early weeks of life through the process of classical conditioning. Since a loss of house training is clearly identifiable by owners, and is one of the distressing clinical signs of CCD, it was decided to use this as a measurement of loss of a previously conditioned behaviour for the purposes of the trial.

The trial was randomised in terms of treatment allocation and was multi-centred, double-blinded and placebo controlled.

The stated inclusion criteria were as follows:

- Over 8 years of age.
- Been in the owner's possession for at least 2 months.
- Been displaying signs of cognitive decline for at least 1 month.
- Behavioural symptoms of cognitive dysfunction:
 - Those signs must include those associated with disorientation.

- In addition the dog must show signs from at least one of the following categories:
 - (i) Changes in social interaction.
 - (ii) Changes in sleep/wake cycle.
 - (iii) Alterations in house soiling incidents.

Age was selected in accordance with the accepted practice of taking 8 years of age as the onset of the “senior” phase of life in domestic dogs but does not take into account breed differences in lifespan and how this may impact on the age of onset of cognitive signs. However, in one study no breed differences in age of onset were identified (Neilson et al., 2001) and in studies by Vetplus Ltd. (2005) and Hills (2000) age related behavioural changes beginning at 7 years and older have been identified in a wide variety of breeds. In fact, recent studies using neuropsychological testing in laboratory beagles, have found learning and memory may begin to decline as early as 6 years of age (Studzinski et al., 2006).

In order to exclude any potential medical factors involved in the presentation of clinical signs all dogs were examined by a veterinary surgeon prior to inclusion in the trial (prior to day –7). This examination consisted of a clinical examination and a blood sample for routine haematology and biochemistry. In addition a brief behavioural history was taken and details were obtained relating to any previous or ongoing medication in relation to the symptoms of canine cognitive dysfunction. Cases were excluded from the trial according to the following exclusion criteria:

- Dogs displaying signs of clinical disease on veterinary examination.
- Dogs showing abnormal results on routine haematology or biochemistry.
- Dogs receiving current treatment in relation to old age behaviour change, e.g. selegiline hydrochloride, nicergoline, propentofylline, nutritional supplements or specific prescription diets for control of the symptoms of old age.
- Dogs exhibiting any behavioural problems relating to disorientation, social interaction, sleep patterns of house training prior to 8 years of age.
- Dogs showing an appreciable level of aggression toward people, which has already resulted in physical injury however minor or has caused the owners reasonable concern that physical injury may occur.

Twenty-four dogs were enrolled in the placebo group and 20 in the treatment group which received the product Aktivait[®] (VetPlus Ltd., Lytham St Anne’s, UK). Dogs were assigned to their respective groups on day –7, although administration of the capsules did not commence until day 0. The veterinary surgeon contacted the trial monitor during the day –7 consultation and the animal was assigned to a group according to a randomised list. The appropriate capsules were then dispensed to the veterinary practice in time for distribution to the owner at the consultation on day 0. In order to ensure that the trial was double-blinded neither the veterinary staff nor the owner was informed as to the identity of the capsule contents. A list of treatment allocations was held at the offices of Vetplus Ltd. and in the event of any need to deblind the owners or veterinary surgeon, for example in a case of suspected adverse reactions or overdose, a telephone number was available for the veterinary practice.

Owners were asked to record the incidence and severity of the behaviours indicative of CCD, over a 56-day period, using a daily questionnaire. The 56 days included a 7-day pre-trial period, a 42-day trial and a 7-day post-trial period. A copy of the survey is available from the corresponding author on request, but the following information regarding specific CCD related behaviours was recorded:

- Number of incidents of lack of recognition of people, other animals or places per day.
- Number of incidents of altered social interaction from or toward the dog per day.
- Number of nights per week that the dog displays restless or broken sleep patterns.
- Number of incidents of inappropriate toileting behaviour per week.
- Number of locations used for inappropriate toileting.
- Number of substrates used for inappropriate toileting.

During the 42-day trial period owners were also asked to record details of the administration of the capsules and account for any that were lost or unsuccessfully administered, giving reasons where possible.

In addition to the daily records owners and veterinary surgeons were asked to give a global assessment score in relation to each of the diagnostic criteria; disorientation, social interaction, sleep patterns and house soiling. The global assessment scores were coded on a numerical scale as insignificant (0), mild (1), significant (2), moderately severe (3), very severe (4). These scores represent a subjective view of the severity of the clinical condition and are based on an assessment of the clinical signs associated with CCD. The incidence scores are taken from the daily records kept by the owners during the trial but the global assessments as to whether those rates of occurrence represent an insignificant, significant or severe level of disorientation, change in social interaction, change in sleep pattern or alteration in house soiling, were made independently by the owner and veterinary surgeon without discussion.

From day 10 of the trial owners and veterinary staff were also asked to record the overall improvement in age related behaviour and quality of life relative to day 0. These scores were coded on a numerical rating scale as significant improvement (3), moderate improvement (2), mild improvement (1), unaffected (0), mild deterioration (−1) and severe deterioration (−2). Owners were asked to make an additional assessment relating to their relationship with the dog relative to day 0 for which the same scoring system was used.

A baseline score for each dog was established after a 7-day pre-trial assessment. This enabled all statistical analysis for the trial to be based on differences in relation to pre-trial data rather than on absolute scores and therefore reduced the potential effect of individual bias. The baseline period was followed by a trial period of 42 days in which the dogs were assigned to treatment groups and given capsules containing either Aktivait[®] or placebo. Finally there was a further 7-day post trial assessment without administration of capsules, in order to assess alterations in behavioural symptoms when treatment was withdrawn. During the total of 56 days the owners attended five face-to-face consultations at the veterinary practice and two phone consultations with the veterinary surgeon or veterinary nurse (see Table 2).

On day −7 the investigator completed a questionnaire which enabled them to collect information regarding the signalment of the animal (age, sex, neuter status and breed) together with details of the onset of clinical signs of CCD and any relevant treatment strategies that had already been instituted. This questionnaire also recorded details of the signs of CCD being exhibited by the dog at the time of the day −7 consultation and the results of the clinical examination and biochemistry and haematology analysis.

During this first face-to-face consultation the owner was given a baseline questionnaire (day −7 to 0) to be completed at home over the next 7 days. On day 0 the owner attended a second face-to-face consultation and the results of the baseline questionnaire were reviewed. Together with the clinical and behavioural information, gathered at the day −7 consultation, the baseline questionnaire enabled the investigator to assess the suitability of the dog for the trial as dictated by the inclusion and exclusion criteria listed above. Provided that the dog was considered suitable for inclusion the owner was provided with 3 weeks' supply of Aktivait[®] or placebo capsules and given day 0–day 21 daily scores questionnaires in order to keep an accurate daily record of their dog's behaviour. On day 10 of the trial the owners received a motivating telephone call from the veterinary practice in order to ensure compliance and to check for any problems. At this time the investigator was asked to complete a phone contact record which included questions relating to the owner's global assessment of their pet's behavioural symptoms and subjective assessments were also made by the owner of the quality of the dog's life, the quality of the relationship between the owner and pet and the alteration in behavioural signs of CCD relative to day 0. On day 21 the third face-to-face consultation was carried out and the owner questionnaire for the period from day 0 to day 21 was reviewed.

Table 2

Trial design—owners were asked to attend face-to-face or telephone consultations on the days indicated in the table

| | Day −7 | Day 0 | Day 10 | Day 21 | Day 28 | Day 42 | Day 49 |
|---------------------------|--------|-------|--------|--------|--------|--------|--------|
| Face-to-face consultation | X | X | | X | | X | X |
| Telephone consultation | | | X | | X | | |

Provided the questionnaire had been satisfactorily completed, and there had been no problems relating to clinical symptoms, a further 3 weeks' supply of Aktivait[®] or placebo was dispensed. The investigator completed a day 21 investigator's questionnaire and recorded information about daily scores together with global scores relative to day 0 of the trial. Global scores were given by both the veterinary surgeon and the owner and overall assessment questions were also asked to both the owner and the veterinary surgeon or nurse relating to overall improvement of behavioural symptoms relative to day 0 of the trial, improvement in the pet-owner relationship and improvement in the pet's quality of life. At the end of the consultation the owner was provided with a questionnaire for the period from day 21 to day 42.

A second telephone consultation was carried out on day 28 and the owner was questioned about any difficulties with the administration of the capsules or the completion of the trial paperwork. The owner was also asked to give global assessment scores for each of the behavioural criteria and subjective assessments of the change in symptoms, quality of life and pet-owner relationship relative to day 0.

During the fourth face-to-face consultation on day 42 the owner's questionnaire covering the period from day 21 to day 42 was reviewed. In addition a further investigator questionnaire was completed in order to obtain information related to the daily scores, global assessments and subjective assessments. No further capsules were dispensed but owners were asked to continue to complete a day 42–day 49 questionnaire on a daily basis.

A final consultation was scheduled for day 49 and during this face-to-face meeting the final owner questionnaire, from day 42 to day 49 was reviewed. A final investigator questionnaire was also completed and the results of an "exit" clinical examination, by the veterinary surgeon, were also recorded. Blood samples were not repeated at the end of the trial.

The daily scores relating to the specific signs of CCD were compared week by week. The data for each week were converted to mean scores for the week. The data actually used in the analyses were the differences compared to the pre-trial week for the daily scores and the first day of the trial for all other measures. The scores for either the pre-trial week (for the daily scores) or the assessments made on day 0 (all other measures) were taken away from the measures made at subsequent consultations. This approach to analysis removes any underlying differences between the dogs and looks at the changes that occur over the course of the trial.

The main analytical method used for the analysis of the results was repeated-measures ANOVA which enabled the analysis of differences between the placebo and Aktivait[®] groups without the problem of non-independence of observations. Unless otherwise stated, variances were homogeneous (as tested by Box's M) and residuals were always approximately normally distributed.

The daily scores for each category were analysed after the removal of severe outliers, as identified using standardised residuals and Cook's distance. The global assessment scores were analysed for improvements in disorientation, social interaction, sleep patterns and housetraining. Analysis was carried out for each criterion separately and they were then analysed in a combined form, with the component scores being added together. The same was done for the subjective assessments of improvement in behaviour, quality of life and owner-pet relationship as made by the veterinary surgeons or nurses and the owners. The justification for doing this was based on Cronbach's α (Bland and Altman, 1997). The assessments of overall improvement in age related behaviour, quality of life and the owner's relationship with the dog were analysed individually and also in a combined form, with the component scores being added together to give an overall improvement as assessed by vets and by owners.

3. Results

A total of 16 out of 24 placebo cases and 11 out of 20 treated cases successfully completed the trial and produced data that was suitable for analysis. The reasons for cases failing to complete the trial were varied and included owners moving away from the area, dogs being removed from the trial for unrelated clinical reasons or being put to sleep for unrelated clinical reasons and owners failing to complete paperwork effectively.

Only a very small number of cases, showing very obvious and consistent unusual behaviour, were removed during the analysis phase. These tended to be placebo cases that showed either an unusually large improvement or an unusually large impairment. It is likely that these cases were responding to some factor outside the control of the study and it is worth noting that the cases from the treated groups were more likely to produce results within the bounds of acceptable variation. A case was only classed as an outlier if it had an absolute standardised residual of more than two on at least four out of six or five out of seven of the sampling dates (depending on the analysis) and the same number of large and obviously unusual Cook's distances. No cases were removed for social interaction, restlessness or house soiling. For the remaining behaviours, three placebo cases were removed for the analysis of recognition behaviour (8.6% of cases); one placebo and one treatment case from the analysis of sleep patterns (5.7%); one treatment case from the analysis of locations for house soiling (2.9%) and one placebo and one treatment case from the analysis of substrates for house soiling (5.7%). For analysis of the global assessment scores and perceived changes in behaviour (both owner and veterinary staff overall assessments) no cases met the criteria for severe outliers and so none were removed.

Analysis of the daily scores from remaining subjects revealed statistically significant differences between the treated and the control groups in relation to sleep patterns ($F_{1, 32} = 7.07$, $p = 0.012$) and recognition ($F_{1, 30} = 4.62$, $p = 0.040$). The results of analysis before the removal of outliers showed a very clear trend in the effect of treatment for all analyses and in no case was the effect size greatly changed by removal of outliers.

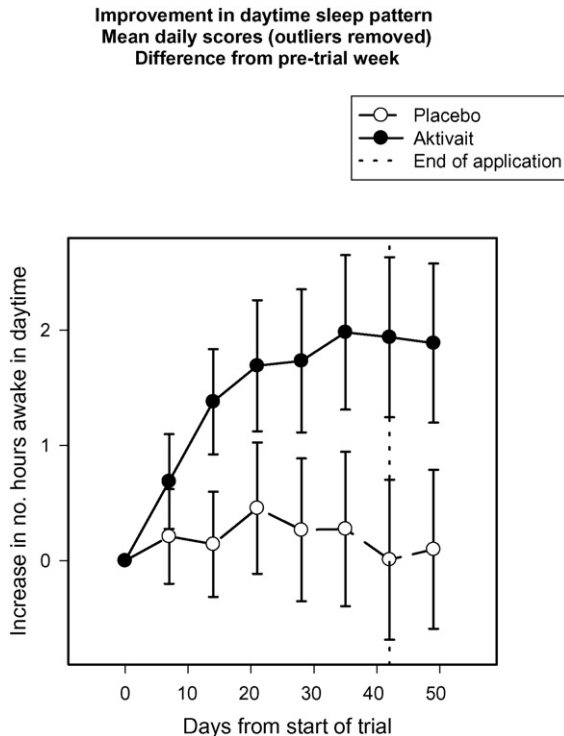


Fig. 1. Improvement in daytime sleep pattern—mean daily scores (outliers removed). Differences from pre-trial week. Error bars represent 95% confidence intervals using the combined standard error.

At the start of the trial all dogs were sleeping for an average of 7 h during the day. The analysis of the daytime sleep pattern scores (after removal of outliers) showed that dogs receiving treatment are likely to be active for an extra 2 h per day—an average improvement of 30% while those dogs receiving placebo showed very little alteration in their daytime sleeping pattern and slept for an average of only 8 min less by the end of the trial (see Fig. 1).

Analysis of the daily scores for recognition behaviour (see Fig. 2) showed that the number of incidents of lack of recognition in dogs receiving treatment were decreased from two to one incident per day while those dogs in the placebo group which showed two incidents per day at the start of the trial showed a reduction of one incident every third day.

Statistically significant differences in the global assessment scores between the treated and placebo groups were recorded for social interaction ($F_{1, 28} = 7.38, p = 0.011$) and house soiling ($F_{1, 28} = 4.74, p = 0.038$).

The house training global assessment revealed that at the start of the trial the average level of house training problems in the treated group was defined as significant while the average level in the placebo group was mild. By the end of the trial the average level in both groups was mild which represented a significant decrease in the treated group (Fig. 3). The difference in the combined global assessment score was also statistically significant between the two groups ($F_{1, 34} = 8.20, p = 0.007$).

The perceived changes in behaviour as assessed by the owners' overall assessment questions revealed statistically significant differences between the two groups in relation to age related behaviour ($F_{1, 34} = 5.91, p = 0.020$), quality of life ($F_{1, 34} = 7.91, p = 0.008$) and the relationship between dog and owner ($F_{1, 33} = 6.98, p = 0.014$) while the overall assessment by the veterinary

**Improvement in recognition behaviour - mean daily scores.
Differences from pre-trial week.**

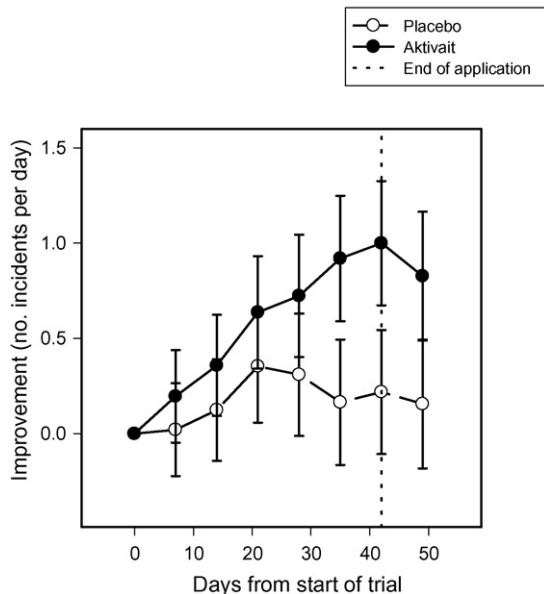


Fig. 2. Improvement in recognition behaviour—mean daily scores. Differences from pre-trial week. Error bars represent 95% confidence intervals using the combined standard error.

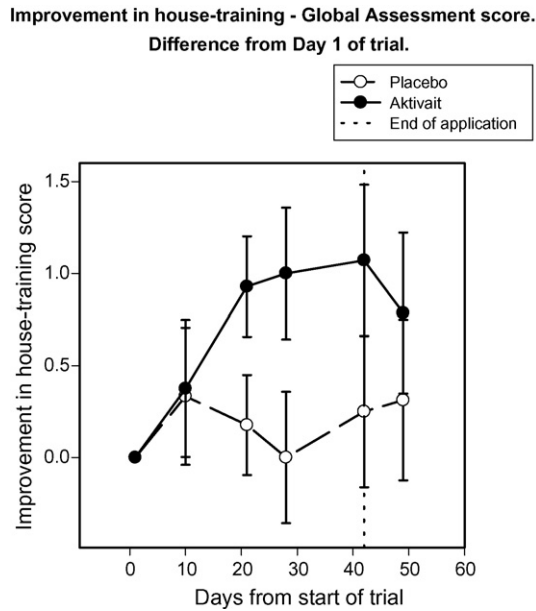


Fig. 3. Improvement in house-training—global assessment score. Differences from day 1 of trial. Error bars represent 95% confidence intervals using the combined standard error.

surgeon or nurse also showed significant differences in relation to age related behaviour ($F_{1, 32} = 8.11, p = 0.008$) and quality of life ($F_{1, 31} = 9.56, p = 0.004$).

4. Discussion

The development of the nutritional supplement used in this clinical trial was based on information gathered from animal and human research. It contains four functional nutrient groups (brain strengthening components, signalling enhancers, metabolic enhancers and antioxidants), which are believed to function in a synergistic fashion and thus provide beneficial effects in the management of the clinical condition of canine cognitive dysfunction (see Table 3).

Table 3
Nutrient groups included in Aktivait[®]

| Constituents of Aktivait [®] | Brain strengthening | Signalling enhancer | Metabolic enhancer | Anti-oxidant |
|---------------------------------------|---------------------|---------------------|--------------------|--------------|
| DHA/EPA | X | | | |
| Phosphatidylserine | X | X | | |
| Acetyl-L-carnitine | | X | X | |
| L-Carnitine | | X | X | |
| CoQ10 | | X | X | X |
| Vitamin C | | | | X |
| Vitamin E | | | | X |
| Selenium | | | | X |
| N-Acetyl cysteine | | | | X |
| α -Lipoic acid | | | | X |

The first group, referred to as the brain strengthening components, contains the fatty acids DHA and EPA, together with the phospholipids, phosphatidylserine. The fatty acids are known to be required for functional optimisation of brain and neural tissue. Deficiencies result in lower learning ability and decreased exploratory behaviour (Lucas et al., 1992). Phosphatidylserine is a major phospholipid in the brain, which facilitates transmission of electrical signals along the nerve cells and has been shown to significantly improve memory retrieval and exploratory behaviour (Maggioni et al., 1990; Vannuchi et al., 1990).

The main constituents offering purported properties of signalling and metabolic enhancement are L-carnitine and acetyl-L-carnitine, both of which are essential in the transportation of long chain fatty acids into the cell. Supplementation, along with α -lipoic acid, significantly improved brain function, memory and activity levels in ageing rats (Lookwood et al., 1994a; Liu et al., 2002a,b). The combination of acetyl-L-carnitine and α -lipoic acid showed a greater effect than either of the components alone (Liu et al., 2002b) and results suggested that feeding a combination of mitochondrial metabolites to old animals may prevent mitochondrial decay in neurons and restore cognitive dysfunction. The paper concluded that these results also suggest that consumption of high levels of mitochondrial metabolites may be an efficient intervention in humans for delaying brain ageing and age associated neurodegenerative diseases. A further study into the combined use of acetyl-L-carnitine and α -lipoic acid in rats also concluded that feeding acetyl-L-carnitine in combination with α -lipoic acid increased metabolism and lowered oxidative stress more than either compound alone (Hagen et al., 2002).

The final group consists of the antioxidants and a combination of six constituents can be found in the product Aktivait[®]. Treatment with certain anti-oxidants including Vitamins C and E, have been shown to protect against age associated oxidative damage to mitochondrial DNA and oxidation of mitochondrial glutathione (Sastre et al., 2000). Vitamin C is an essential water-soluble dietary antioxidant, which also regenerates Vitamin E and glutathione and protects flavonoids, while Vitamin E is a lipid soluble antioxidant, which is important in its action against lipid peroxidation. Another constituent is *N*-acetyl cysteine (NAC), which is a precursor of glutathione and is a major water-soluble antioxidant. Levels are significantly reduced under oxidative stress conditions and supplementation provides consistent increases in cellular and plasma glutathione levels. Co-enzyme Q10 is a lipid soluble antioxidant, which regenerates Vitamin E and is a significant antioxidant. It is also a T-cell stimulant and has tumour cell control properties (Hagen et al., 2002; Lookwood et al., 1994b). The final two ingredients with antioxidant properties are selenium and α -lipoic acid. The latter is an endogenous antioxidant, which has been shown to recycle other enzymatic antioxidants and have synergistic action when administered in combination with acetyl-L-carnitine (Lookwood et al., 1994a; Liu et al., 2002b).

The discrepancy between the number of cases enrolled on the trial and the number that produced data, which could be included in statistical analysis resulted from a variety of factors. Some cases failed to complete the trial due to dog related factors, such as removal from the trial due to unrelated clinical illness or death of the dog from unrelated medical causes. This is a particular risk with trials, which have specifically selected an ageing population. Human related factors, such as moving house during the duration of the trial and failure to complete the paperwork correctly, were also encountered.

The most significant results in terms of daily scores related to disorientation and sleep patterns but the results of analysis before the removal of outliers, showed a very clear trend in the effect of treatment for all analyses and results. It would have been beneficial to increase the number of cases enrolled on the trial and the duration of the trial in order to investigate these effects further. One of the limitations of clinical trials such as this one is the recruiting and retention of sufficient

numbers of cases and one of the important aims of future studies into the effectiveness of nutritional supplementation in cases of canine cognitive dysfunction should be to recruit a larger sample size.

In view of the progressive and irreversible nature of the condition of canine cognitive dysfunction one important clinical consideration is the need for continuing nutritional supplementation. In this study a post trial period of 7 days was included and a relapse in all symptoms was particularly marked in the treatment group after withdrawal of supplementation on day 49. However, extension of the post trial period would be beneficial in order to investigate this effect in more detail.

The clinical signs of cognitive dysfunction, which include disorientation, changes in social interaction, alterations in sleep patterns and loss of previously learned behaviours such as house training, have serious welfare implications for the dog but also have a significant impact on the owner and their relationship with their pet. The significant results in relation to global assessment of social interaction and house soiling are particularly important in terms of the owner's perception of their pet's response to treatment and this is reflected in the owners' overall assessment scores which revealed statistically significant differences between the treated and the placebo groups in relation to age related behaviour, quality of life of the dog and the relationship between dog and owner. The veterinary surgeon or nurse was independently asked to rate the level of improvement in relation to age related behaviour and quality of life of the dog and the statistically significant results between the two groups further confirm the positive benefits of nutritional supplementation in the management of canine cognitive dysfunction.

5. Conclusions

In spite of its relatively small scale, the results of this trial indicate a clear beneficial effect of nutritional supplementation on some aspects of behaviour associated with canine cognitive dysfunction. Significant positive changes occurred in both the objective behaviour of dogs suffering from CCD as well as in the perception of the dogs' condition by people in regular contact with them. These findings lend support to the clinical practice of nutritional supplementation in cases of canine cognitive dysfunction. In combination with behavioural and environmental management and psychopharmacological intervention, nutritional supplementation has a potentially valuable role to play in maximising the benefits of therapy in terms of increased quality of life. Key to this benefit may be the early detection of symptoms of cognitive dysfunction thereby enabling dogs to receive the most appropriate veterinary care.

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