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The chew was found to be well-tolerated and similar in terms of aspect and acceptability to the current one. All but one mobility criteria significantly improved after 30 days, including the ability to walk, climb, stand, interact with people, or the way of walking.

**Keywords:** movoflex; movoflex advanced; dogs' mobility support; tolerance in dogs; movoflex efficiency; dog mobility; krill; hyaluronic acid; owner perception; movoflex soft chew.

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# Adding Krill Oil and Low Molecular Weight Hyaluronic Acid to Movoflex® Soft Chews Helps Improve Dogs' Mobility

## Movoflex® Advanced Soft Chews on Dogs' Mobility

Nicolas CS <sup>α</sup>, Schreiber P <sup>σ</sup>, Rème CA <sup>ρ</sup>, Lopez J <sup>ω</sup> & Ereau C <sup>¥</sup>

**Abstract-** Movoflex® Soft Chew (Virbac, USA) is a nutritional supplement to support dogs' mobility. It contains a unique combination of five ingredients: eggshell membrane, hyaluronic acid (HA) of high molecular weight, *Boswellia serrata* extract, astaxanthin, and vitamin D3. Krill oil and low molecular weight HA are now added to the supplement. The tolerance of this advanced formula was first evaluated in dogs. The aspect, palatability of the chew, and effectiveness on mobility criteria were then assessed by sixty-nine dog owners who were regular users of the current Movoflex® Soft Chews.

The chew was found to be well-tolerated and similar in terms of aspect and acceptability to the current one. All but one mobility criteria significantly improved after 30 days, including the ability to walk, climb, stand, interact with people, or the way of walking.

Therefore, the new Movoflex® Advanced Soft Chew (Virbac, USA) is well tolerated and more effective than the current formula.

**Keywords:** movoflex; movoflex advanced; dogs' mobility support; tolerance in dogs; movoflex efficiency; dog mobility; krill; hyaluronic acid; owner perception; movoflex soft chew.

### 1. INTRODUCTION

Osteoarthritis (OA) is a common disorder in humans and animals, and its prevalence increases with age. It can reach more than 80% of animals eight years of age or older [1]. It is a debilitating and painful disease, involving local tissue damage as well as local and systemic inflammatory responses. It leads to decreased mobility and impacts the quality of life of both dogs and owners [2–4]. Preventing cartilage damage and oxidative stress, and maintaining a balanced immune response, are therefore key to disease prevention and to slowing down its progression. Management of OA in dogs usually involves nonsteroidal anti-inflammatory drugs despite their side effects. Many efforts have been put over the last decades into finding new active ingredients and nutraceuticals to maintain joint health and mobility [3,5].

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Movoflex® Soft Chew (Virbac, USA) is a nutritional supplement that can help support dogs' mobility and joint health [2]. It contains a unique combination of ingredients that are well tolerated by dogs and that have shown effectiveness in supporting joint health, either individually or in combination: eggshell membrane (ESM), astaxanthin, hyaluronic acid (of high molecular weight), *Boswellia serrata* extract, and vitamin D3 [2]. Now, the advanced formula also contains hyaluronic acid (HA) of low molecular weight and krill oil as a source of omega-3 fatty acids.

Eggshell membrane contains different molecules found in joints, including glucosamine and glycosaminoglycans, elastin, collagen, and hyaluronic acid. It also contains different amino acids (mainly proline, glutamic acid, and glycine) that can help support protein synthesis [6]. Eggshell membrane has shown beneficial effects on joints and mobility, in humans with osteoarthritis [7–14] and in dogs [2,15,16].

Astaxanthin is a naturally occurring carotenoid with potent antioxidant properties (more powerful than other carotenoids and 500 times more powerful than vitamin E) [17–19]. Astaxanthin limits the production of mitochondrial reactive oxygen species (ROS) and modulates their effects, scavenging free radicals and decreasing the development of nitric oxide [18,19]. Astaxanthin has also been shown to stimulate the immune response in animals and humans [20,21]. The astaxanthin in Movoflex® Soft Chews comes from *Haematococcus pluvialis*, a unicellular microalga, one of the safest and richest sources of natural astaxanthin [18,19].

*Boswellia serrata* extracts, from the resin of the tree, have been used for joint health for a long time, notably in ayurvedic medicine. Several studies have shown its good tolerance and beneficial effects on joints in humans and animals [22,23], including in dogs [24]. Boswellic acids like 3-O-Acetyl-11-keto-beta-boswellic acid (AKBA) and 11-keto-b-boswellic acid (KBA) would indeed help control the immune system by inhibiting the 5-lipoxygenase (5-LOX) and would help improve pain and joint function [22,23]. Other mechanisms may also be involved.

Cholecalciferol is the inactive form of vitamin D3. It requires sequential hydroxylations in the liver and kidney to reach its physiologically active form (calcitriol or 1,25-dihydroxyvitamin D3). It is a steroidal hormone known to regulate calcium and phosphorus homeostasis in the body, as well as bone metabolism, among other properties [25,26]. The blood level of vitamin D is positively correlated with good musculoskeletal health and negatively correlated with OA or mobility reduction [25–29]. It has been shown in some studies that vitamin D supplementation can help patients with OA [25,30,31]. In dogs, vitamin D supplementation can help maintain bone health [26,29].

Hyaluronic acid (HA) is a large glycosaminoglycan consisting of repeating disaccharide units of N-acetylglucosamine and glucuronate. It is found in several tissues, including in the articular cartilage and synovial fluid. It is highly involved in the lubrication and viscoelasticity of the synovial fluid and plays a significant role in joint mobility [32,33]. Therefore, administering HA has shown beneficial effects in OA dogs [32,33]. Since OA is associated with HA degradation over time, the HA administered was, mainly, of high molecular weight (MW) and usually injected directly into the joint [33]. However, oral forms can also be used with good bioavailability and efficacy [34,35]. A study has shown that HA of high MW can be found in dogs' joints after oral administration [36]. On the other hand, lower MW HA, which should have a higher oral bioavailability than the high MW HA [34,37], could bring the elements required for the HA synthesis and help initiate the restorative processes [33,38]. A recent study in an animal model even showed that the lower molecular weight HA would be more effective than the higher molecular weight HA for cartilage repair [38].

Krill are shrimp-like crustaceans that are small (about the size of a paperclip) but form abundant and dense swarms in polar seas. The oil extracted from the main Antarctic species, *Euphausia superba*, is naturally rich in omega-3 polyunsaturated fatty acids (or PUFAs), mainly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) [39]. Contrary to what is found in fish oil (mainly triacylglyceride-bound PUFAs), PUFAs from krill oil come primarily in the phospholipid (PLs)-bound form. Phosphatidylcholine is a major PL in krill oil. On top of bringing essential nutrients like choline, PLs also seem to be a more effective delivery form of PUFAs, with higher bioavailability [39,40]. The health benefits of krill oil have been demonstrated in several studies, including those focusing on joint health [39,41,42]. It has also been suggested that PLs, like those in krill oil, could increase the bioavailability of astaxanthin and hyaluronic acid [43,44]. Combining these three ingredients has shown beneficial effects on joint and inflammation in different studies [45,46] and is now commercially available for humans (FlexPro MD®, Valensa).

The krill used to make the oil in Movoflex® advanced Soft Chews (Virbac, USA) is fished using an eco-harvesting technology to harvest krill in a sustainable and environmentally friendly way. This technology reduces the risk of by-catch of other species to almost zero.

We hypothesized that adding krill oil and a lower molecular weight HA could increase the performance of Movoflex® Soft Chews in dogs and remain well tolerated. We first tested its tolerance, in kennel dogs, before testing its acceptability and perception of effectiveness on mobility, with dog owners already using the current Movoflex® Soft Chews.

## II. MATERIAL AND METHODS

### • Tolerance

The tolerance of the chew was tested in 16 beagle dogs (6 months to 2 years old, > 5 kg) receiving either one or five times the daily recommended number of chews, according to Virbac's standard procedures. The project was approved by the Virbac Ethical Committee and by French Authorities under authorization number 20210111113595530#28915.

Eight dogs received one Movoflex® Soft Chew size S per day (as recommended) for small dogs < 18 kg (1x group), and eight dogs received five chews per day (5x group), during 28 days. Another group of four dogs did not receive any chew (control group). A 14-day acclimation phase was included before administering the chews on Day 0. Dogs were housed in pairs and fed Veterinary HPM® Adult Neutered Dog (Virbac). They were observed daily, and a complete clinical examination, including body weight and body temperature measurements, was performed once a week from Day -14 to 28. Food consumption was checked daily. An adjustment of the food ration (-10g of kibbles daily) was made for the dogs receiving five chews per day (6.27 kcal/chew). Feces were examined and scored daily based on a simple scale (1- molded stools, 2- not molded stools, 3- liquid stools) during the whole study period. Blood samples were taken at the start and the end of the study for standard hematology [RBC, HGB, HCT, MCV, MCH, MCHC, RETIC, WBC, NEU, EOS, BASO, LYM, MONO and PLT - ProCyteDx Hematology Analyser (IDEXX)] and blood chemistry [BUN; CREA; TP; ALB; GLOB; GLU; ALKP; ALT; AST; Calcium; Chloride; Potassium; Magnesium; Sodium; Phosphate - Catalyst Chemistry Analyser (IDEXX)] analysis. Urine samples were also taken on fasted and sedated animals by ultrasound-guided cystocentesis in females and by vesical catheterization in males, when possible (no empty bladder) at the beginning and the end of the study for pH analysis. Urinary pH was measured with a pHmeter (Mettler Toledo Seven Multi pH meter) quickly after urine collection.

- Perceived effectiveness

Movoflex® Advanced Soft Chews were provided to 78 owners of dogs, in the USA, already using Movoflex® Soft Chews before the study. Selected dogs had to be five years or older and included 26 small dogs weighing less than 18 kg (<40 lbs), 26 medium dogs between 18 and 36 kg (40-80 lbs) and 26 large dogs weighing more than 36 kg (>80 lbs). The participants tested the chew over 30 days (1 chew per day - chew size according to body weight: 2g, 4g, or 6g, respectively). The participants had to fill out an online survey on Days 0, 7, and 30 to assess the product's physical properties (versus the current Movoflex® Soft Chews), palatability, reactions of the dog, and perception of effectiveness on their dog's mobility.

Every day, the owner had to score the reaction of the dog when given the chew, according to the following scale: 1- the dog ate the chew all at once; 2 - the dog ate part of it, stopped, then ate the rest; 3 - the dog ate some of it only; 4 - the dog played with it but did not eat; or 5 - the dog would not touch it spontaneously. To assess their dog's mobility, the owners had to describe five parameters:

- Dog's interaction with family and friends (1 - Happy and playful/Jumped around; 2 - Happy but reserved; 3 - Neutral; 4 - Sad, but reacted when called; 5 - Sad and didn't react much when called; 6 - Tended to isolate from others; 7 - Became aggressive when disturbed)
- Dog's ability to stand up after resting/lying down for some time (1 - Normal, stood up without difficulty; 2 - Slow, with some difficulty; 3 - Slow, with a lot of difficulty; 4 - Needed help getting up)
- Dog's ability to walk (1 - Normal, walks with ease; 2 - Normal, but needs to stop often; 3 - Difficult, needs to be encouraged; 4 - Difficult, refuses to walk)
- The way the dog walks (1 - Walks normally; 2 - Walks with stiffness; 3 - Unsteady when walking; 4 - Walks with lameness)
- Dog's ability to climb steps (1 - Normal; 2 - Slow; 3 - Made regular stops; 4 - Needed to be encouraged; 5 - Needed help/holding; 6 - Did not climb steps)

They also had to score from 0 (not difficult) to 10 (very difficult) the dog's ability/difficulty to walk, and the dog's ability/difficulty to stand up after some rest.

**Statistics:** Due to the ordinal nature of the data, Friedman's tests were used with the results obtained on Days 0, 7, and 30, for each mobility criteria. In case of significance ( $p < 0.05$ ), Wilcoxon's signed-rank tests were used, using Bonferroni's correction to compare the data obtained on Day 0 versus Day 7 or Day 30 ( $p < 0.025$  for significance).

### III. RESULTS

- Tolerance

All dogs in the group receiving five chews per day (5x group) ate all their chews every day, while five dogs in the group receiving one chew per day (1x group) did not consume it every day (no forced administration). These same dogs also generally had a low food consumption, suggesting a general lack of appetite. Four of these dogs (who did not eat their chew more than 15 times) were excluded from the analysis while the other one was kept for analysis (only one day with no consumption). All dogs remained healthy throughout the study, and no product-related clinical signs were observed. Mild signs (ocular discharge, mild induced cough, licking granuloma) could be observed in some dogs, in all groups, before and after product administration, and were therefore unrelated to product administration. Digestive signs (vomiting or bloody stools) were observed twice in the 1x group animals during the administration period, with no link to product administration. Indeed, these signs can be observed sporadically in dogs with no clinical significance and were not observed in the 5x group.

The mean dog body weight and body temperature remained stable during the administration period (Supp. Table 1).

Concerning blood analysis (hematology, biochemistry and electrolytes), all mean values were within physiological ranges except for the globulin and MCHC, which were slightly lower than the reference range in all groups and at both time points (Supp. Table 2-5). All values remained pretty stable throughout the study. Some individual values could be slightly out of range but with no link to product administration (observed before and after the chew administration and in all groups).

Urine samples could be taken at both time points (Day -14 and Day 28) for the four control dogs, three dogs in the 1x group (excluding those who did not eat the chew every day), and six dogs in the 5x group. In all individuals, urine pH was between 5.11 and 6.82, with no relevant change between Day -14 and 28 (Supp. Table 6).

Therefore, no product-related clinical signs were observed during the study. Movoflex® Advanced Soft Chews are well tolerated by dogs, even when given five chews per day for 28 days, instead of one as recommended.

- Perceived effectiveness

Among the 78 owners recruited on Day 0, 69 completed the study by filling in the questionnaires on the three days (Days 0, 7, and 30). The 69 owners included 21 owners of small dogs (<18 kg or <40 lbs), 25 owners of medium dogs (18-36 kg, or 40-80 lbs), and 23 owners of large dogs (>36 kg or >80 lbs).



Most owners reported giving the chew by hand (57% and 51% on Day 7 and Day 30, respectively) or on the bowl with food (51% and 41%, respectively). The remaining owners mostly gave it on the floor (13% and 9%, respectively).

During the trial, depending on the days, between 90% and 96% of dogs completely ate the chew. In 89% of cases (over a total of 2070 chew presentations), the dogs ate the chew all at once, and in 4% of cases, the dog ate part of it at first and the rest later (total consumption in 93% of cases). The chew was partially taken in 1% of cases, and in 7% of cases the dog would not take the chew spontaneously. Five dogs (7%: three small dogs and two medium dogs) partially took the chew or refused to take the chew spontaneously more than 50% of the time and were excluded from the analysis on mobility criteria (but were kept for the general appreciation).

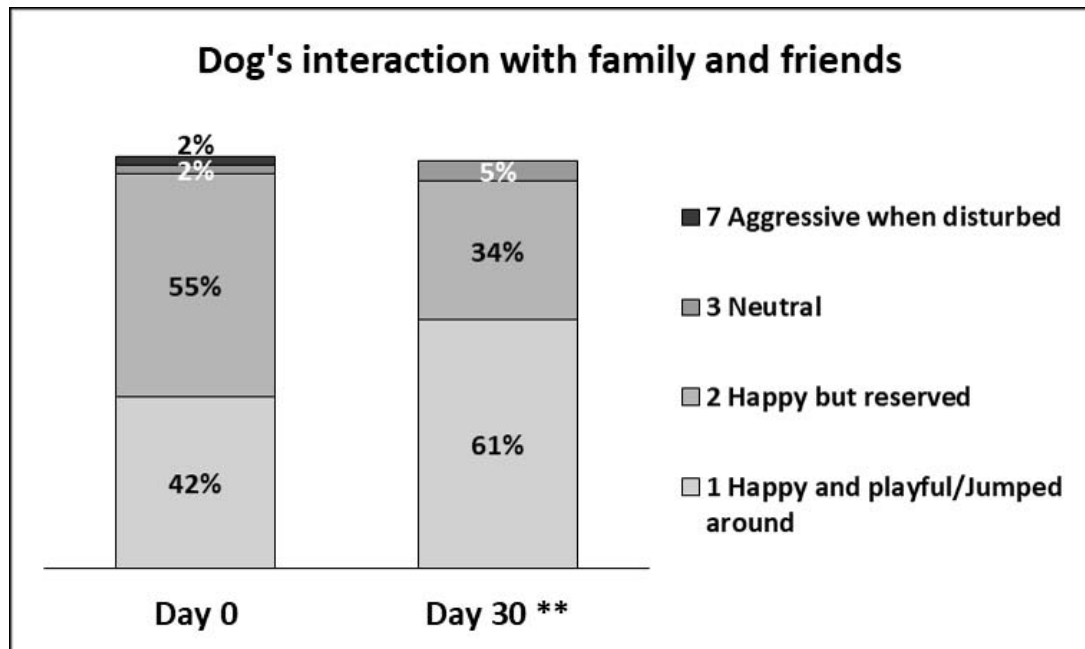
By Day 30, most owners thought the tested chew was as easy (83%), or easier (13%) to give as/

than the previous Movoflex® Soft Chew and that palatability was similar (75%) or even better (16%).

Most owners also found the size, smell, color or shape of the new test chew were similar to the previous product they used.

Concerning the dog's mobility, 63% of the 64 owners analyzed considered there was an improvement in their dog's ability to move around by Day 30 (34% on Day 7).

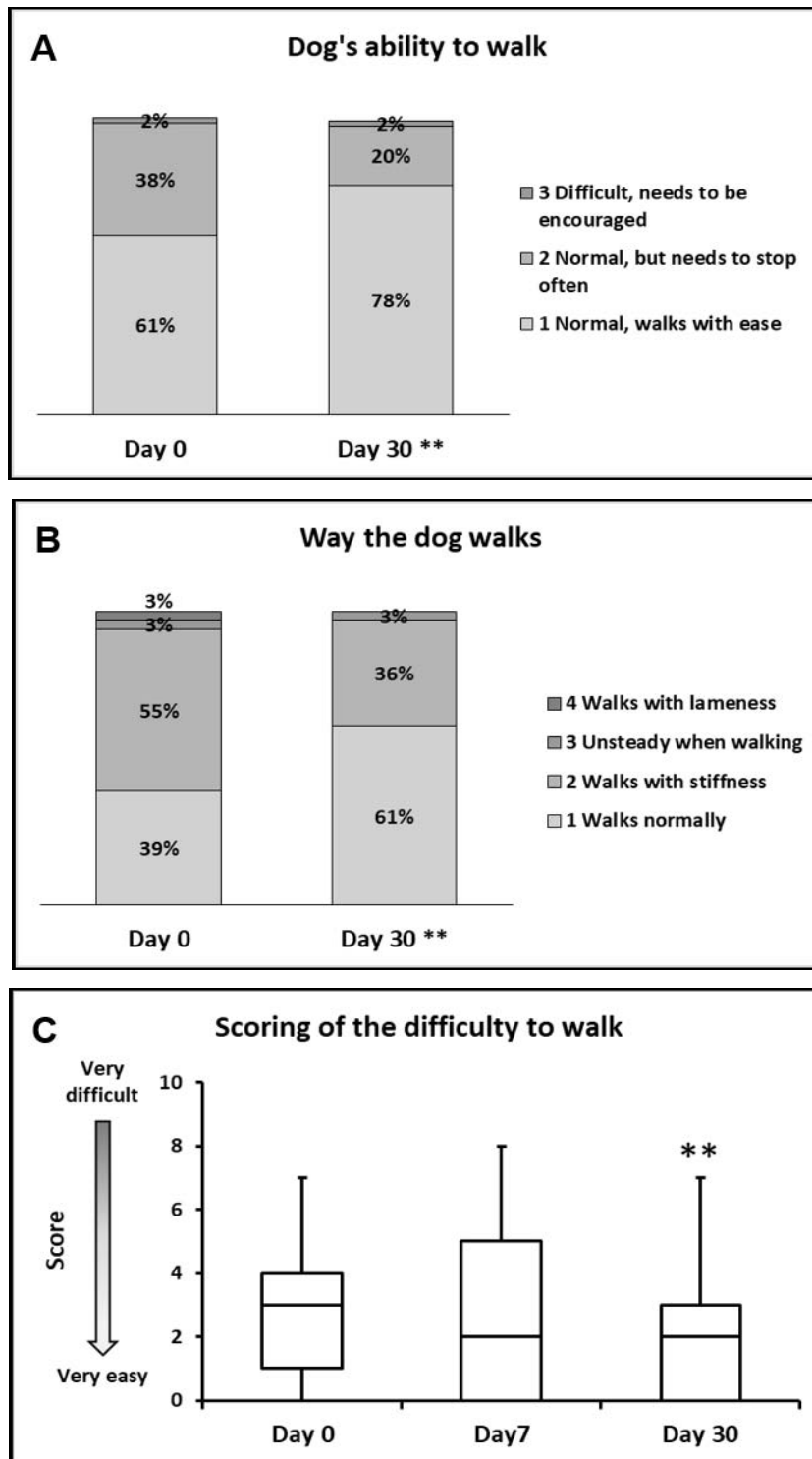
- Interaction with family and friends: on Day 0, 55% of owners considered their dogs happy but reserved (score 2), while 42% considered them happy and playful (score 1). On Day 30, this repartition changed to 34% and 61%, respectively (Figure 1). The appreciation (score) given for this parameter was significantly different by Day 30 compared to Day 0 ( $p < 0.01$ ), with 23% of owners giving a better (lower) score at the end of the study.



**Figure 1:** Dog's interaction with family and friends. The percentage of owners describing their dogs as: 1 - Happy and playful/Jumped around; 2 - Happy but reserved; 3 - Neutral; 4 - Sad, but reacted when called; 5 - Sad and didn't react much when called; 6 - Tended to isolate from others; or 7 - Became aggressive when disturbed, are depicted in this graph on day 0 (D0) and day 30 (D30). No owners reported their dogs to be 4, 5 or 6. A significant improvement was observed between Day 0 and Day 30 (\*\*,  $p < 0.01$ , Wilcoxon Signed rank test,  $n = 64$ ).

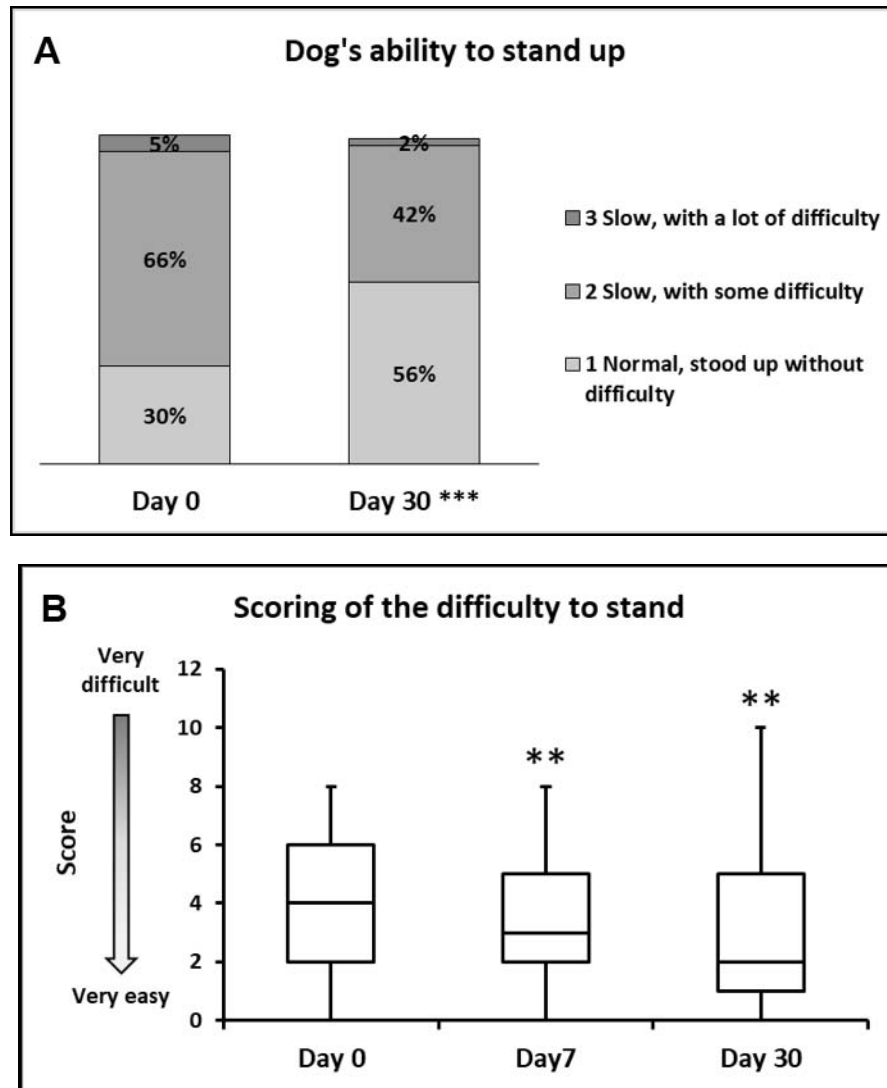
- Dogs walking ability
  - Dog's ability to walk: most owners found their dog's ability to walk normal and with ease on Day 0 (61%) while some said their dogs needed to stop often (38%). By Day 30, more owners said their dogs walked with ease (78%, Figure 2). The appreciation (score) given for this parameter significantly improved by Day 30 ( $p < 0.01$ ), with 19% of owners giving a better appreciation by then.
  - The way of walking was described to involve stiffness by 55% of owners on Day 0, while only 39% said their dogs walked normally. By Day 30, more owners said their dogs walked normally (61%, Figure 2). The appreciation of this parameter significantly improved as of Day 7 ( $p < 0.01$ ; 20% of owners gave a better appreciation) and was further improved by Day 30 (31% of owners gave a better appreciation).

- When asked to score their dog's ability/difficulty to walk from 0 (very easy) to 10 (very difficult), the median (Q1-Q3) score significantly decreased from 3 (1-4) to 2 (0-3) between Day 0 and Day 30 ( $p < 0.01$ , Figure 2).



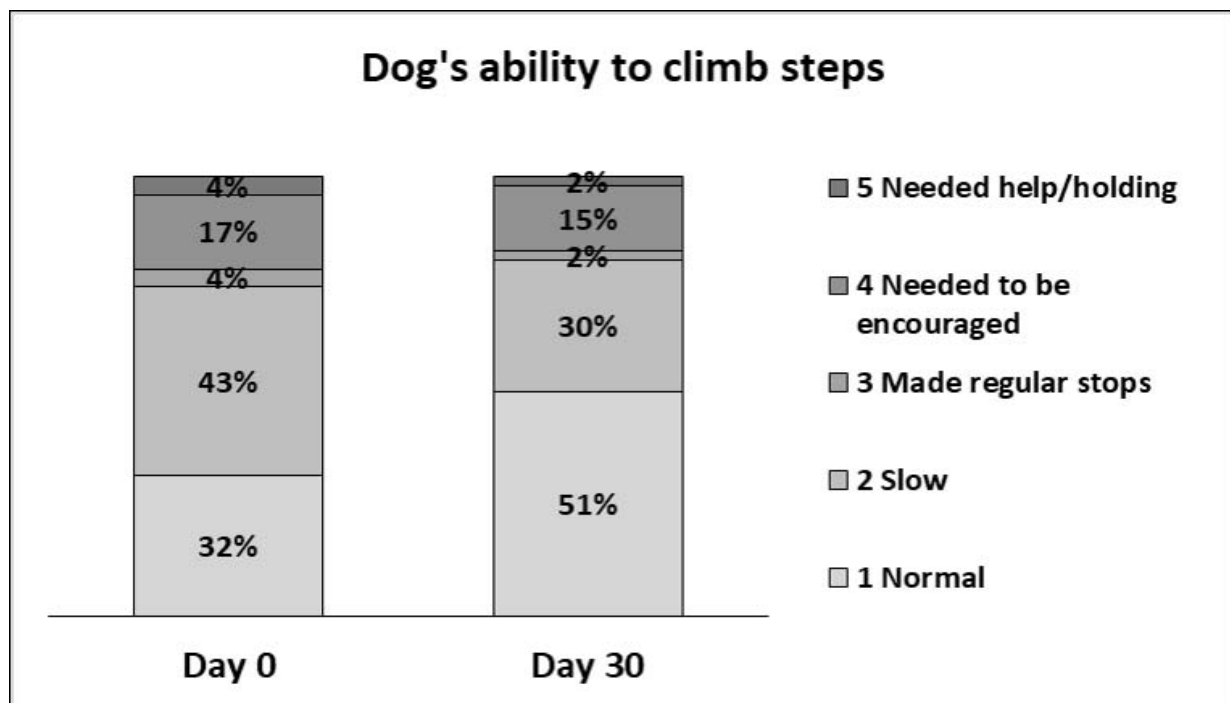
**Figure 2:** Dog's walking ability. A: Percentages of owners describing their dogs ability to walk as: 1 - Normal, walks with ease; 2 - Normal, but needs to stop often; 3 - Difficult, needs to be encouraged or 4 - Difficult, refuses to walk. B: Percentages of owners describing their dog's way of walking as: 1 - Walks normally; 2 - Walks with stiffness; 3 - Unsteady when walking or 4 - Walks with lameness. C: Box plot representing the median, Q1-Q3, min-max scores given by the owners for the dog's ability/difficulty to walk (on a scale from 0 - very easy to 10 - very difficult). For A,B,C: \*\*,  $p < 0.01$ .

- Dog's ability to stand up
- The dog's ability to stand after resting/lying also improved significantly as of Day 7 ( $p < 0.025$ ; 20% of owners gave a better appreciation) and was further improved by Day 30 (31% of owners gave a better appreciation.  $p < 0.001$ ). Indeed, on Day 0, 66% of owners described their dog's ability to stand as slow, with some difficulty, while on Day 30, this number fell to 42%, and more owners felt their dogs could stand up with no difficulty (Figure 3)
- When asked to score their dog's ability/difficulty to stand from 0 (very easy) to 10 (very difficult), the median (Q1-Q3) scores given by owners significantly decreased from 4 (2-6) to 3 (2-5) and 2 (1-5) on Days 0, 7, and 30, respectively ( $p < 0.01$ , Figure 3).



**Figure 3:** Dog's ability to stand up after resting/lying down for a period of time. A: Percentages of owners describing their dog's ability to stand as 1 - Normal, stood up without difficulty; 2 - Slow, with some difficulty; 3 - Slow, with a lot of difficulty; 4 - Needed help getting up. \*\*\*,  $p < 0.001$  B: Box plot representing the median, Q1-Q3, min-max scores given by the owners for the dog's ability to stand up after resting (on a scale from 0 -very easy to 10 - very difficult). \*\*,  $p < 0.01$ .

- Dog's ability to climb steps: no significant improvement was observed for this parameter, based on the owner's appreciation (score). However, most owners described their dogs as slow when climbing steps on Day 0 (43%), while on Day 30, most owners (51%) described their dog's ability to climb as normal (Figure 4).
- Finally, among the 69 owners who tested the new product, 87% said they would definitely (65%) or probably (22%) buy the new Movoflex® Advanced Soft Chews. This purchase intention rate increased to 90% (70% definitely and 20% probably) if it is recommended by a veterinarian.



**Figure 4:** Dog's ability to climb steps. Percentages of owners describing their dog's ability to climb steps as 1 - Normal; 2 - Slow; 3 - Made regular stops; 4 - Needed to be encouraged; 5 - Needed help/holding; or 6 - Did not climb steps.

#### IV. DISCUSSION

The advanced formula of Movoflex® Soft Chews (Virbac, USA), with low molecular weight HA and krill oil, was tested for its tolerance, palatability, and perceived effectiveness in dogs.

The tolerance evaluation showed that the chew was well tolerated by healthy dogs, even with five chews per day for 28 days. The dogs remained in good health, and no clinical signs linked to the product were observed. There was also no change in blood parameters or urine pH.

When the new chew formula was given for one month to 69 dogs who were previously taking the current Movoflex® Soft Chews, it was completely consumed in 93% of cases, and 91% of owners found the acceptability of the new chew was similar or even better than the previous one. According to dog owners, no change in the shape, aspect, or odor was observed compared to the current Movoflex® Soft Chews.

Since the dogs who tried the new formula previously received Movoflex® Soft Chews regularly, their mobility issues were mild but could still be improved. After seven days of daily administration, some mobility criteria were already improved, like the way of walking, and the ability to stand after lying down for some time. At this stage (Day 7), 34% of owners considered their dog's mobility had improved. By Day 30, all mobility criteria but one (ability to climb steps) significantly improved, and 63% of owners noticed the improvement in their dog's mobility.

These data suggest that adding krill oil and hyaluronic acid (HA) of low molecular weight to a supplement already containing ESM, astaxanthin, vitamin D3, *Boswellia serrata* extract, and HA of high molecular weight (Movoflex® Soft Chew) can improve its efficiency.

This finding could be explained by the omega-3 PUFA found in krill oil that can improve the bioavailability of astaxanthin and HA [43,44], enhancing their effects, on top of the known beneficial effects of omega-3 PUFA on joint health [41,42,47].

Hyaluronic acid can be found in joints, particularly in the synovial fluid. It has been shown that the concentration and molecular weight (MW) of HA in animals with OA were decreasing and that administering HA could help in maintaining joint health [33]. The high MW HA would be of interest to preserve the viscoelasticity of the synovial fluid, and the lower MW HA could help modulate the inflammation process and participate in cartilage repair [33,38].

Owners' rating of improvement remains a subjective way to evaluate the efficacy of supplements, and the findings need to be further supported by proper clinical studies involving veterinarians. However, owners remain the best persons to appreciate the progress of their dog's mobility on a day-to-day basis. Such studies, with less limited inclusion criteria and constraints, can include more participants and are performed in circumstances of evaluation closer to what is happening in real life.



Despite these limitations, the data suggest that the complex blend of ingredients found in Movoflex® Advanced Soft Chews can help improve dogs' mobility. Adding low MW HA and krill oil to the original formula led to improved benefits.

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## CONFLICT OF INTEREST

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- Abbreviations**  
 AKBA: 3-O-Acetyl-11-keto-beta-boswellic acid  
 BASO: Basophils  
 DHA: Docosahexaenoic Acid  
 EOS: Eosinophils  
 EPA: Eicosapentaenoic Acid  
 ESM: Eggshell Membrane  
 HA: Hyaluronic Acid  
 HCT: Hematocrit;  
 HGB: Hemoglobin;  
 KBA: 11-keto-b-boswellic acid  
 LOX: Lipoxygenase  
 LYM: Lymphocytes  
 MCV: Mean corpuscular volume  
 MCH: Mean corpuscular hemoglobin  
 MCHC: Mean corpuscular hemoglobin concentration  
 MONO: Monocytes  
 MW: Molecular Weight  
 NEU: Neutrophils  
 OA: Osteoarthritis  
 PL: Phospholipid  
 PLT: Platelets  
 PUFA: Polyunsaturated Fatty Acids  
 RETIC: Reticulocytes  
 ROS: Reactive Oxygen Species  
 RBC: Red blood cells  
 WBC: White blood cells

Supp. Table 1: Body weight and temperature (mean and SD are reported)

Group		Body weight (kg)		Body temperature	
		D0	D28	D0	D28
Control (n=8)	Mean	9,93	9,97	38,4	38,2
	SD	2,33	2,36	0,3	0,3
1x group (n=4)	Mean	11,52	11,53	38,1	38,3
	SD	3,31	3,26	0,2	0,2
5x group (n=8)	Mean	11,63	11,81	38,8	38,4
	SD	3,60	3,67	0,4	0,3

*Supp. Table 2:* Red blood cell count and parameters (mean and SD are reported).

Group		RBC (10x6.0/ $\mu$ l)		HGB (g/dl)		HCT (%)		MCV (fl)		MCH (pg)		MCHC (g/dl)		RETIC (/ $\mu$ l)		RETIC (%)	
		D-7	D28	D-7	D28	D-7	D28	D-7	D28	D-7	D28	D-7	D28	D-7	D28	D-7	D28
Control (n=8)	Mean	7.4	7.5	17.2	17.0	52.5	53.1	71.5	71.5	23.5	22.9	32.8	32.0	37325	35225	0.51	0.48
	SD	0.1	0.7	0.3	1.2	1.4	3.1	2.4	3.1	0.5	0.8	0.4	0.4	21255	2173	0.30	0.04
1x group (n=4)	Mean	7.5	7.4	17.5	17.2	53.7	53.2	72.1	71.8	23.5	23.2	32.6	32.3	70025	80125	0.93	1.06
	SD	0.6	0.6	1.5	1.3	3.9	3.6	2.3	2.2	1.3	1.1	0.8	0.6	26644	31916	0.33	0.34
5x group (n=8)	Mean	7.0	7.2	16.5	16.8	50.6	52.1	72.8	72.9	23.6	23.5	32.4	32.3	52400	50450	0.76	0.70
	SD	0.7	0.6	1.7	1.2	4.1	3.7	3.0	2.0	0.5	0.7	1.1	0.4	15737	18654	0.23	0.21
Ref. values		5.48.7		13.420.7		38.3 - 56.5		59 - 76		21.9 - 26.1		32.6 - 39.2					

RBC: Red blood cells; HGB: Hemoglobin; HCT: Hematocrit; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; RETIC: Reticulocytes.

*Supp. Table 3:* White blood cell count (mean and SD are reported).

Group		WBC (1000/ $\mu$ l)		NEU (/ $\mu$ l)		NEU (%)		EOS (/ $\mu$ l)		EOS (%)		BASO (/ $\mu$ l)		BASO (%)		LYM (/ $\mu$ l)		LYM (%)		MONO (/ $\mu$ l)		MONO (%)		PLT (1000/ $\mu$ l)	
		D-7	D28	D-7	D28	D-7	D28	D-7	D28	D-7	D28	D-7	D28	D-7	D28	D-7	D28	D-7	D28	D-7	D28	D-7	D28	D-7	D28
Control (n=8)	Mean	9,7	8,9	7593	5551	79	64	290	420	3	4	0	0	0	0	1353	2416	14	27	400	496	4	5	252	198
	SD	2,5	2,3	1931	874	3	9	247	332	2	3	0	0	0	0	524	1083	3	6	171	297	2	2	79	34
1x group (n=4)	Mean	8,4	9,0	5407	5635	66	63	437	790	5	9	0	0	0	0	2032	2079	23	23	505	449	7	5	272	247
	SD	2,3	2,2	1001	1292	10	4	409	334	3	3	0	0	0	0	1435	779	9	5	116	57	2	2	50	31
5x group (n=8)	Mean	10,5	11,7	7184	7786	69	66	285	363	3	3	0	0	0	0	2504	3028	23	27	472	477	5	4	293	277
	SD	1,6	3,2	844	3011	8	7	223	100	2	1	0	0	0	0	1149	768	7	6	158	147	2	1	105	101
Ref. values		4,9-17,6		2940-12670				70-1490				0- 100				1060-4950				130-1150				143 -448	

WBC: White blood cells; NEU: Neutrophils; EOS: Eosinophils; BASO: Basophils; LYM: Lymphocytes; MONO: Monocytes; PLT: Platelets

*Supp. Table 4:* Blood chemistry parameters (mean and SD are reported).

Group		BUN (mmol/L)		CREA (mmol/L)		TP (g/L)		ALB (g/L)		GLOB (g/L)		GLU (mmol/L)		ALKP (IU/L)		ALT (IU/L)		AST (IU/L)	
		D-7	D28	D-7	D28	D-7	D28	D-7	D28	D-7	D28	D-7	D28	D-7	D28	D-7	D28	D-7	D28
Control (n=8)	Mean	5,1	5,1	54	53	55	55	33	32	22	23	5,2	5,1	33	33	29	31	22	27
	SD	0,6	0,6	11	8	3	3	1	1	2	2	0,9	0,8	2	6	13	17	3	6
1x group (n=4)	Mean	5,3	5,9	59	60	53	54	32	32	21	22	5,4	5,7	35	47	21	22	22	23
	SD	0,3	0,6	9	7	6	6	6	5	1	1	0,5	0,5	14	21	7	6	3	5
5x group (n=8)	Mean	5,2	5,5	57	57	55	55	32	31	23	23	5,7	5,5	39	39	33	34	24	27
	SD	0,8	0,8	13	10	3	3	2	2	3	2	0,3	0,4	16	13	12	14	5	6
Ref. values		3,2 -10,3		44- 103		54-76		28-43		24 -43		3,2 -7,0		14 - 147		25-122		14-59	

BUN: Blood ureanitrogen; CREA: Creatinine; TP: Total protein; ALB: Albumin; GLOB: Globulin; GLU: Glucose; ALKP: Alkaline Phosphatase; ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase

*Supp. Table 5:* Blood electrolytes (mean and SD are reported).

Group		Ca (mmol/L)		Cl (mmol/L)		K (mmol/L)		Mg (mmol/L)		Na (mmol/L)		Phos (mmol/L)	
		D-7	D28	D-7	D28	D-7	D28	D-7	D28	D-7	D28	D-7	D28
Control (n=8)	Mean	2,5	2,5	114	112	4,2	4,0	0,8	0,8	148	148	1,2	1,3
	SD	0,1	0,1	2	1	0,3	0,1	0,1	0,1	2	0	0,2	0,2
1x group (n=4)	Mean	2,5	2,5	114	114	4,1	4,1	0,8	0,8	148	149	1,3	1,2
	SD	0,1	0,1	1	2	0,2	0,2	0,1	0,1	1	1	0,2	0,1
5x group (n=8)	Mean	2,5	2,4	114	113	4,1	4,2	0,7	0,7	147	148	1,2	1,3
	SD	0,1	0,1	1	2	0,2	0,2	0,0	0,0	1	1	0,2	0,1
Ref. values		2,1 - 2,9		106 - 120		3,9 - 5,8		0,7 - 1,1		142 - 153		0,9 - 1,7	

*Supp. Table 6:* Urine pH (mean and SD)

Group		Urine pH	
		D-14	D28
Control (n=4)	Mean	5.91	5.62
	SD	0.73	0.28
1x group (n=3)	Mean	5.74	5.66
	SD	0.33	0.34
5x group (n=6)	Mean	5.82	5.55
	SD	0.26	0.41
Ref. values		5,3 – 7,0	