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Neurogenic inflammation in the upper digestive tract of the mule duck: effect of a chemical algogen and force-feeding

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Abstract 1. The objectives were to quantify the presence of neurogenic inflammation in 4 regions of the upper digestive tract of anaesthetised ducks (post-pharynx, pseudo-crop, transition between the pseudo-crop and the proventriculus, and proventriculus) after application of HCl stimulation of up to 4 M in the pseudo-crop.

2. The second objective was to quantify the presence of neurogenic inflammation in the same digestive tract regions as mentioned above during 4 feeding periods of *foie gras* production (rearing, preparation to force-feeding, and second and last meals of the force-feeding period).

3. Extravasation increased above a HCl stimulation threshold of 2 M. Furthermore, more extravasation was observed in the proventriculus compared to the other regions ($P < 0.001$).

4. Highest extravasation responses were observed in the proventriculus and the pseudo-crop at the end of the preparation period, and in the proventriculus after the second forced meal, compared with the rearing period ($P < 0.01$), with a return to rearing level at the end of force-feeding.

5. Such a kinetic could be indicative of a relative mildness of the irritant components associated with this feeding practice.

INTRODUCTION

In the last 10 years, force-feeding production to obtain *foie gras* has been questioned in terms of animal welfare. Very strong criticism and concerns are currently raised at this type of production because it involves catching the bird, introducing a tube into its oesophagus, and forcing a large amount of food through this tube twice daily for almost two weeks (Guémené *et al.*, 2001). Several studies have considered the technical (Rousselot-Paillet, 1997), nutritional, histological (Wyers and Vuillaume, 1996), biochemical (Leprettre *et al.*, 2002) and pathological (Babile *et al.*, 1996; Hermier *et al.*, 1999) consequences of force-feeding. Very little information is available on the potential negative effect of the

force-feeding procedure due to inflammation in the upper digestive tract walls where the tube and forced bolus are introduced (Servière *et al.*, 2003b).

An irritant stimulus (mechanical or chemical) tends to cause neurogenic inflammation (review by Bircklein and Schmelz, 2008). In mammals, the main components of neurogenic inflammation include local vasodilatation and increased vascular wall permeability, leading to plasma leakage into the tissues (i.e. extravasation), and local histamine and proinflammatory cytokine release. Subsequently, inflammation can enhance responses to different stimuli, including nociceptive stimuli (Lamb *et al.*, 2007).

Numerous studies on mammals have used mustard oil or capsaicin as algogens to trigger

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neurogenic inflammation (Wesselmann and Lai, 1997; Grant *et al.*, 2005). The topical application of these agents led to local blood flow increase (hyperaemia) and vascular permeability, thus triggering the extravasation response and sensitising nociceptors (review by Richardson and Vasko, 2002). Quantification of neurogenic inflammation can be achieved by measuring its major component, an increase in vascular permeability, in any type of tissue (Carr and Wilhelm, 1964; Saria and Lundberg, 1983). Most often, the technique used to demonstrate vascular permeability and plasma leakage involves a preliminary intravenous injection of a physiological dye with a large molecular weight (e.g. Evans blue), followed by application of the irritant stimulus. This has been demonstrated in various types of tissue such as rodent gastrointestinal tract (Figini *et al.*, 1997) and bladder (Jasmin *et al.*, 1998). With poultry, mustard oil stimulation has been used for chicken skin or wattle (Gentle and Hunter, 1993) and with the oesophagus of ducks (Servière *et al.*, 2003a, 2004).

The link between nociceptive sensations and gastrointestinal syndromes such as inflammation is not well understood for domestic birds. Repeated forced introduction of a tube and large food bolus into the pseudo-crop of the duck, associated with force-feeding, is likely to stretch the walls of the pseudo-crop; and, in addition, due to the possible abrasive component of the maize bolus, exert a mechanical irritant effect upon the upper digestive tract walls. If force-feeding induces visceral neurogenic inflammation, it could further lead to visceral discomfort due to long-lasting inflammation. To our knowledge, apart from some preliminary results (Servière *et al.*, 2003b, 2004), no data on visceral neurogenic inflammation regarding force-feeding are available for ducks.

The aim of the first experiment was to quantify the degree of extravasation in the upper digestive tract of the duck after application of an exogenous irritant stimulus, known to induce neurogenic inflammation in other species. This first experiment allowed investigation of differential extravasation responses in 4 different regions of the duck upper digestive tract (post-pharynx, pseudo-crop, transition between the pseudo-crop and the proventriculus, and proventriculus). However, one of the problems with investigation of neurogenic inflammation in birds is the absence of a response to the most widely-used chemical agents that induce a mammalian neurogenic inflammatory response (Geisthovel *et al.*, 1986). Capsaicin has been reported to be ineffective in ducks (Geisthovel *et al.*, 1986), due to the lack of an avian equivalent of the mammalian vanilloid receptor VR1

(Clapham, 1997). Hence, use of HCl as a chemical trigger may be appropriate to induce neurogenic inflammation in the upper digestive tract, as already demonstrated for the oesophagus of guinea pigs (Rouget *et al.*, 2004).

The aim of the second experiment was to quantify the degree of neurogenic inflammation in the same 4 parts of the upper digestive tract as for HCl stimulation, during the 4 feeding periods in *foie gras* production.

MATERIALS AND METHODS

Rearing and feeding ducks

In total, 200 male mule ducks from a heavy strain (Gourmaud selection 49, La Seigneurtière 85260 Saint André, France: hybrid of Muscovy drake (MMG, *Cairina moschata*) and heavy Pekin female (PKL, *Anas platyrhynchos*)) were grown under natural conditions of light and temperature at the Unité Expérimentale des Palmipèdes à Foie Gras. Briefly, ducks were housed collectively with access to an open-range area from hatching to 13 weeks of age (4.2 ducks/m² until 4 weeks of age, and 2.8 ducks/m² thereafter). From 0 to 3 weeks of age, the ducks had free access to a diet containing 11.8 MJ ME and 175 g crude protein/kg. From 4 to 5 weeks of age, a growing diet containing 11.7 MJ ME and 145 g crude protein/kg was provided *ad libitum*. From 6 to 12 weeks the same diet was provided, but daily intake was reduced to avoid excessive fatness (average of 220 g/day/bird). The following period, i.e. preparation to force-feeding, began at 13 weeks of age in an open area. During the 6 consecutive days of preparation, the amount of food (i.e. 4 mm diameter Landal factory pellets, 40280 Haut-Mauco, France) was increased by an amount of 30 g/day/bird. At the end of this period, the ducks' voluntary food intake was 400 g/day/bird. The aim of the preparation to force-feeding was to increase the volume of the compartments of the upper digestive tract (pseudo-crop and proventriculus), and to increase enzyme synthesis and metabolic adaptation. From 14 weeks of age, and during the 13 d of the force-feeding period, ducks were randomly allocated to colony cages (5 per cage, 1445 cm² per duck) and received two identical forced meals per day delivered through a stainless-steel tube using a hydraulic pump device. The procedure was designed to ensure that the handling protocol applied to the ducks was standardised. Each meal consisted of a carbohydrate-rich diet composed of 35% mashed maize, 25% maize grain (14.2 MJ ME, 77 g protein/kg) and 40% water. To improve digestibility, the maize was given as a mixture of whole and crushed grains hydrated with water. The total amount of food was progressively

increased from 480 to 900 g/day. Water was available *ad libitum*. Water dispensers allowed ducks to immerse the head into water. No prophylactic medication was provided before or during the force-feeding period.

Ducks used for HCl stimulation were grown in the same rearing conditions as force-fed ducks, from hatching to 14 weeks of age, but without the period of preparation. From the 14th week of age, they were housed in colony cages (5 per cage) identical to those used for the force-fed ducks until the experiment one week later. Meanwhile, they were given the grower diet provided at 13 weeks of age and free access to water.

Chemical stimulation in the upper digestive tract of ducks

For the validation of the presence of extravasation in the upper digestive tract of the ducks after an irritant stimulation, birds were randomly taken from the colony cages at the age of 15 weeks, put into a box and transferred to a different room. There, they were submitted to gaseous anaesthesia (isomolar mixture of N₂O/O₂ as gaseous support and 2% isoflurane, Forene, Abbott, 94150 Rungis, France), laid in a supine position on the operation table, kept warm with a thermostatic blanket and maintained under spontaneous respiration using tracheal intubation until euthanasia. At 10 minutes before HCl stimulation, 2 mL saline (9 g NaCl/l) containing Evans blue dye was injected intravenously (40 mg/kg live weight, RAL-reactives, 75015 Paris, France). HCl irritant nociceptive stimulation was achieved by introducing into the digestive tract a commercial standard wad of cotton wool (25 mm diameter) moistened with 5 mL saline containing different HCl concentrations ($n=4$ per concentration): 0, 0.1 M, 0.25 M, 0.5 M, 1 M, 2 M, 4 M (HCl 37% aqueous solution, Fischer Scientific, 67400 Illkirch, France). In order to prevent direct irritant stimulation of the walls of the upper parts of the oesophagus, the cotton wool was placed down to mid-level of the pseudo-crop via a standard force-feeding tube (diameter 28 mm).

Force-feeding stimulation

Different periods of the feeding regime were studied ($n=8$ ducks per period): the rearing period at the end of the 12th week; the preparation to the force-feeding period at the end of the 13th week; the second meal and the last meal of the force-feeding period. The ducks received an intravenous bolus of 2 mL saline containing Evans blue (40 mg/kg live weight, RAL-reactives, 75015 Paris, France), either immediately after access to food, in order not to modify

spontaneous food intake due to handling (preparation period); or 10 min before the force-fed meal (2nd or last meal).

Euthanasia

One hour after stimulation (HCl or force-feeding), a sub-lethal dose of sodium pentobarbital (100 mg/kg live weight) was injected intravenously. The ducks were immediately perfused via an intracardiac route to flush out the blood and fix the tissues (4 l saline –1% paraformaldehyde solution (VWR-Prolabo, 94126 Fontenay-sous-Bois, France) at 38°C; followed by 5 l saline –4% paraformaldehyde solution at 4°C). Immediately after complete perfusion, the upper part of the digestive tract was removed, cleaned and divided into 4 sections: post-pharynx (part of the oesophagus corresponding to the length of the tube introduced, i.e. 120 mm); pseudo-crop; narrow part corresponding to the transition region between the pseudo-crop and the proventriculus; and proventriculus.

Measuring of the extravasation response

The tissue samples were processed using the method described by Saria and Lundberg (1983) and McMahon *et al.* (1984): each piece of tissue was initially wiped and weighed, then dried (48 h at 50°C), weighed again and immersed in formamide (Merck, 94120 Fontenay-sous-Bois, France) for 48 h at 50°C to extract Evans blue dye. Absorption of the solution was measured with a spectrophotometer (emission at 680 nm) (Carr and Wilhelm, 1964). Concentrations were expressed in µg per g of dry tissue.

Statistical analyses

A tissue sample from one duck (from the preparation period) was discarded due to the presence of candidosis *post mortem* (no clinical signs were evident in the live duck).

When a normal distribution of the residuals was verified using a Kolmogorov-Smirnov test, data were analysed by the MIXED model procedure of Statistical Analysis Systems Institute (SAS® 9.1.3, SAS Institute, Cary, NC, USA) with *post-hoc* comparison of the estimates (*t*-test based). The model included either the concentration of HCl as a fixed effect; the fixed effect of the region (post-pharynx, pseudo-crop, transition region, proventriculus); or the interaction between the concentration (or period of feeding) and the region, with the repeated factor region. When normal distribution of the residuals was not verified, log, square root or inverse transformations were used. Because normal distribution of the residuals was not verified for the

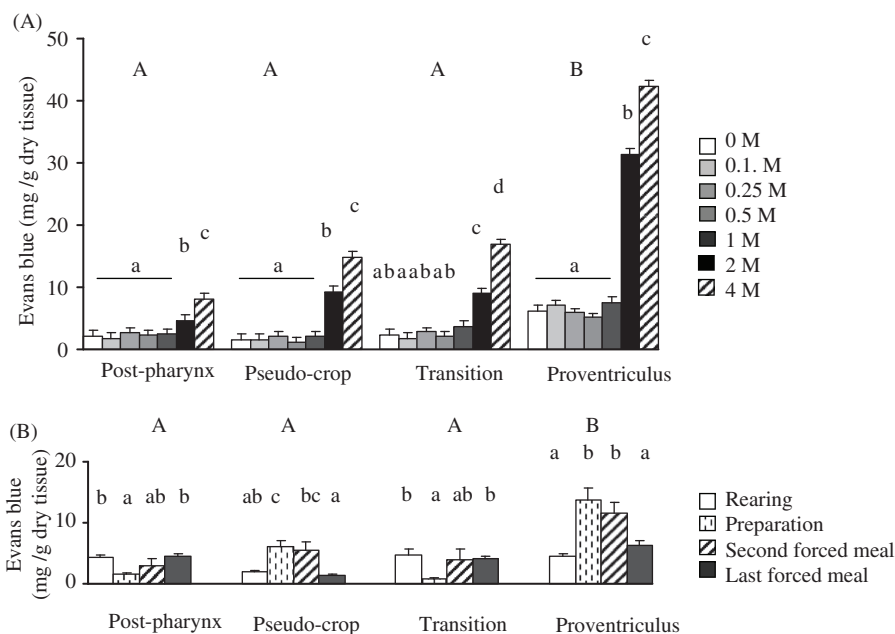


Figure 1. Extravasation responses (Evans blue dye concentration, $\mu\text{g/g}$ dry tissue) in the upper digestive tract of the duck after (A) HCl stimulation to the pseudo-crop ($n=4$ for each concentration) and (B) different feeding periods: rearing, preparation to force-feeding, second forced meal and last forced meal ($n=8$ ducks for each feeding period). Extravasation was measured according to region (post-pharynx, pseudo-crop, transition region and proventriculus). Values with different superscripts a,b or A, B differ within a region or between regions ($P \leq 0.05$). HCl stimulation: back-transformed data, Least Squares means \pm se. Mixed model procedure. Feeding procedure: means \pm SE. Kruskal-Wallis one-way analysis of variance by ranks and Mann-Whitney test post-hoc comparison.

force-feeding experiment even after transformations (Kolmogorov-Smirnov test), non-parametric tests were performed (Siegel and Castellan, 1988) using the Statistica 5.1 (StatSoft, Tulsa, OK, USA). To test for a regional effect, a Friedman two-way analysis of variance by ranks (for k related samples) was used. For *post-hoc* comparison, a Wilcoxon matched pair test was conducted. For comparison of the feeding periods, a Kruskal-Wallis one-way analysis of variance by ranks (for k independent samples) was used. For *post-hoc* comparison, a Mann-Whitney U test was used. Because it is not possible to test the interaction between the feeding period and the region of the digestive tract with non-parametric analysis, and because the regional effect was significant, the effect of the feeding period was studied region by region. A Kruskal-Wallis one-way analysis of variance by ranks (for k independent samples) was used to analyse the overall feeding period effect for each region separately. If significant, a *post-hoc* comparison Mann-Whitney U test was done. Least squares means \pm SE are presented when proc MIXED was conducted, while means \pm SE are presented for non-parametric analysis.

Ethical guidelines

The present study was carried out according to French legislation on animal experimentation, and with the authorisation of the French Ministry

of Agriculture (Ministère de l'Agriculture, DDSV, Direction de la Santé et de la Protection Animale, licence n° 78-52). French ethical procedures are in line with the European Convention for the Protection of Vertebrates used for Experimental and other Scientific Purposes (European Directive 86/609).

RESULTS

Extravasation response to HCl

Stimulation with different HCl concentrations produced a dose-related response ($P < 0.001$) as measured by the Evans blue concentration in the tissues (Figure 1). A higher Evans blue concentration was evident with concentrations of 2 and 4 M HCl, compared with more neutral conditions: 0.1, 0.25, 0.5 and 1 M HCl ($P \leq 0.001$).

A regional effect ($P \leq 0.001$) was evident after HCl stimulation. The proventriculus was the most sensitive to HCl stimulation, showing the highest Evans blue concentration compared with the post-pharynx, pseudo-crop, and transition region ($P \leq 0.001$). Evans blue concentrations were lower in the pseudo-crop and post-pharynx than in the transition region ($P \leq 0.01$) ($\mu\text{g/g}$ dry tissue, square root transformation with back-transformed data within brackets; post-pharynx: $1.8 (3.2) \pm 0.06$; pseudo-crop: $1.9 (3.6) \pm 0.06$; transition region: $2.1 (4.6) \pm 0.06$; proventriculus: $3.5 (12.5) \pm 0.06$).

An interaction between dose and region ($P \leq 0.001$) was found. A large increase in extravasation was observed for HCl concentrations higher than 1 M in the 4 regions of the upper digestive tract (Figure 1) compared with saline. Moreover, between 1 M and 2 M, a more than 4-fold increase was observed for the proventriculus and pseudo-crop compared with only a two-fold increase for the post-pharynx and transition region. In addition, for all HCl concentrations (from neutral conditions to 4 M), ducks had a higher concentration of Evans blue in the proventriculus compared with the post-pharynx, the pseudo-crop and the transition region ($P \leq 0.001$).

Extravasation response to force-feeding

No significant effect of the feeding period was evident. A regional effect ($P \leq 0.001$) was observed for Evans blue concentrations in ducks submitted to force-feeding: extravasation was greater in the proventriculus than in the post-pharynx, the pseudo-crop and transition region ($P \leq 0.001$) ($\mu\text{g/g}$ dry tissue, post-pharynx: 3.4 ± 0.37 ; pseudo-crop: 3.7 ± 0.55 ; transition: 3.5 ± 0.55 ; proventriculus: 8.9 ± 0.93).

However, when the effect of the feeding period was considered region by region (Figure 1), a significant effect of the feeding period was evident in the post-pharynx ($P \leq 0.05$), in the pseudo-crop ($P \leq 0.001$), in the transition region ($P \leq 0.05$) and in the proventriculus ($P \leq 0.001$). In the post-pharynx, a lower extravasation response was evident during preparation, compared with the rearing period and the last forced meal ($P \leq 0.01$). The same pattern was observed in the transition region: a lower extravasation response was observed during the preparation period compared with the rearing period and the last forced meal ($P \leq 0.001$). In the pseudo-crop, a greater extravasation response was evident during the preparation period compared with the rearing period or the last forced meal ($P \leq 0.001$). In addition, a higher extravasation response was observed after the second compared with the last forced meal ($P \leq 0.01$). In the proventriculus, a higher extravasation response was evident during the preparation period ($P \leq 0.001$) and after the second forced meal ($P \leq 0.01$) compared with the rearing period and the last forced meal.

DISCUSSION

The first experiment demonstrated that after chemical stimulation, an extravasation response, an indicator of neurogenic inflammation, was evident with differing intensities according to the

region in the duck upper digestive tract. This dose effect had a threshold of 2 M HCl. This pattern of response has similarities with the dose effect reported for graded irritant stimulations in rats (Lamb *et al.*, 2003) and ducks (Servière *et al.*, 2004). In addition, a considerable regional effect was observed: the extravasation response was two-to-four fold higher in the proventriculus than in the post-pharynx (oesophagus), pseudo-crop and transition regions. In the proventriculus, the extravasation response was particularly pronounced for stimulations with 2 M HCl and above, but the saline response was already more pronounced compared with other regions. The proventriculus appeared to be the most reactive region of the upper digestive tract when submitted to the irritant action of a chemical agent. This observation confirms earlier results obtained using mustard oil, the highest quantitative extravasation response being observed in the proventriculus (Servière *et al.*, 2004). This higher sensitivity can be partly explained by differences in histological structures and functions. In contrast to the upper parts of the digestive tract, which are mainly involved in the transit of food (the post-pharynx and the transition region between the pseudo-crop and the proventriculus region) or transient storage of food (the pseudo-crop); the proventriculus, or the anterior glandular stomach, is primarily involved in digestive processes (Farner, 1960; Sibley, 1977). One of its functions is to synthesise and secrete digestive enzymes: the inner layer is a secretory epithelium with tubular unilobular glands, opened into collecting tubules radially arranged around a collecting chamber which communicates with the lumen of the proventriculus (Farner, 1960). In addition, studies using rodents and dogs (Fioramonti and Bueno, 1984; Anzueto Hernandez *et al.*, 1986) showed that the digestive processes induced a transient high energy metabolic rate and triggered local increase in blood flow (hyperaemia). A similar mechanism is likely to occur in the proventriculus of the duck. This may partly account for the greater extravasation responses even under basal conditions. In contrast, a lower extravasation response was observed in the post-pharynx, pseudo-crop and transition regions. With HCl stimulation in the pseudo-crop, a small quantity of acid may have migrated into the upper part of the digestive tract, the post-pharynx. Moreover, in the pseudo-crop, epithelial glands produce a lubricating fluid which is likely to exert an additional protective coating effect due to the storage function in this part of the digestive tract (Farner, 1960; Hodges, 1974).

During the force-feeding procedure, a regional effect but not a period effect was observed. However, when studying the period effect in

specific regions of the tract, higher extravasation responses were evident in the pseudo-crop and proventriculus at the end of the preparation period, when the animals voluntarily ingested large amounts of food over a short period of time and without any physical constraint, and after the second forced meal (i.e. at the beginning of the force-feeding period). These results raise several questions. In particular, it could be expected that the greatest extravasation response would be at the end of the force-feeding period, not at the end of the preparation period. One explanation for the higher extravasation during preparation to force-feeding, compared with rearing, is that food pellets spontaneously ingested during this period have a repetitive mechanical irritant effect due to their abrasive characteristics (volume and texture). During the preparation for force-feeding, the quantity of food provided to each duck increased rapidly. Combined with the moderate food restriction during the end of the rearing period, this led to a spontaneous increase in the amount ingested and the rate of intake without any physical constraint, mainly due to the competition between the animals, and to the large eating capacity of male mule ducks (up to 750 g daily: Guy *et al.*, 1997). This technique allowed preparation for the force-feeding without any handling being involved. The extravasation response observed after the second forced meal may result from the force-feeding procedure, or from the procedures in the preparation period. In addition, the fact that the extravasation response was intermediate between the preparation period and the last forced meal period could reflect the beginning of the inflammation resorption. The second result to emphasise is the return, by the end of the force-feeding period, to initial values similar to those obtained during the rearing period. While extravasation is a sign of local neurogenic inflammation, the decrease in the extravasation response in the proventriculus and the pseudo-crop at the end of the force-feeding period could be interpreted as the result of an active biochemical process initiated to resolve inflammation (review by Serhan *et al.*, 2007), in order to get the tissues back to their initial physiological status. Such a kinetic in the upper digestive tract of the duck could be indicative of the relative mildness of the irritant components associated with this feeding practice.

In contrast to the pseudo-crop and proventriculus, less extravasation was evident in the post-pharynx and the transition region during the preparation period, compared with the rearing period or the last forced meal. However, the fact that extravasation was less than during the rearing period was unexpected because no effect, or a small increase, in extravasation would

have been expected in these two regions, as observed for the pseudo-crop or proventriculus. It should be noted that these two regions are only involved in the transit of food, and they have neither a storage function as in the case of the pseudo-crop, nor a digestive one as in the proventriculus (Farner, 1960; Hodges, 1974). This decrease in extravasation response in the transition region and post-pharynx during preparation compared with rearing is difficult to explain. Nevertheless, it may result from a protective mechanism against abrasion occurring over the 6 days of preparation, which induces considerable cornification within the transition and post-pharynx regions. The pseudo-crop (oesophagus) and transition region are composed of thick stratified squamous epithelium with mucous and secretory glands, and have a different structure to the pseudo-crop and proventriculus (Hodges, 1974). Cornification is an adaptation process which occurs in the mucous membrane of the upper digestive tract of birds, and has been reported after certain feeding practices, such as an excess in tocopherol (vitamin E) (Wang, 1993), or a confrontation with irritant factors such as nematode infestation (Fatihu *et al.*, 1992).

In addition to the abrasive effects of pellets or a food bolus, it can be argued that mechanical stimulation, such as excessive distension, leads to nociception, as reported with colonic distension in humans (review by Julius and Basbaum, 2001). However, in humans submitted to upper gastrointestinal endoscopy without anaesthesia, unpleasant sensations have been reported but no nociception (Rambaud, 2005). In addition, in the case of force-feeding, the anatomical organisation of the upper digestive tract renders excessive stretching very unlikely. This is due to longitudinal foldings of the pseudo-crop and the absence of cartilaginous rings along the whole tract. Indeed, like most water birds, ducks are equipped with an upper digestive tract adapted for storage and swallowing of rather large prey such as fish and shellfish (Farner, 1960; Sibley, 1977). A neurophysiological approach, with the study of c-Fos activation during force-feeding procedures, suggested that force-feeding generated mild nociceptive information encoded in the nervous system (Servière *et al.*, 2003a, 2004).

A question can be raised concerning the comparison between HCl stimulation and force-feeding. During the force-feeding procedure, the highest extravasation observed in the proventriculus and pseudo-crop corresponded to the extravasation obtained with HCl stimulation between 1 M and 2 M. The extravasation observed in the post-pharynx and transition zone were in the range of the extravasation obtained with HCl

concentration between 0 M and 1 M. However, a basal Evans blue extravasation was observed with a neutral emulsion and during rearing, probably due to permanent basal permeability of digestive tract walls. This could be due to ongoing basal exchanges through digestive tract blood vessels, rather than the result of an inflammatory process *per se*. The effects of HCl stimulation represent an acute response to chemical stimulation; whereas force-feeding generates longer-term responses to mechanical stimulation. Different types of stimulation (chemical and mechanical) can lead to the same final response (i.e. neurogenic inflammation and sensitisation of C-fibre endings), although signal-transduction mechanisms might be different (review by Christianson *et al.*, 2009). Nociceptors and transmitters implicated in neurogenic inflammation have not been studied in ducks. However, the process of neurogenic inflammation has been extensively studied in rats (review by Richardson and Vasko, 2002) and, in a few cases, in chickens and pigeons. Indeed, polymodal nociceptors implicated in sensing physical and chemical stimuli, and sensitised by inflammation, have been identified in numerous locations in the chicken, such as the beak (Dubbeldam *et al.*, 1995), the tarsometatarsus skin (Gentle and Tilston, 2000) and leg articulation (Gentle *et al.*, 2003).

CONCLUSION

Indirect comparison between degrees of extravasation after chemical nociceptive stimulation and the force-feeding procedure suggests that the mechanical insult on the oesophagus walls, potentially associated with the preparation to force-feeding and force-feeding procedure, is moderate. This could explain the return to a low neurogenic inflammation level at the end of the force-feeding period. However, further studies are needed to determine whether force-feeding, or experimental irritant chemical stimulation, induce visceral nociception or discomfort.

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