



High pressure inactivation of selected avian viral pathogens in chicken meat homogenate



Roman Buckow^a, John Bingham^{b,*}, Susie Daglas^b, Sue Lowther^b, Rachel Amos-Ritchie^b, Deborah Middleton^b

^a Commonwealth Scientific and Industrial Research Organisation, Food and Nutrition, 671 Sneydes Road, Private Bag 16, Werribee, VIC 3030, Australia

^b Commonwealth Scientific and Industrial Research Organisation, Australian Animal Health Laboratory, 5 Portarlington Road, Geelong, VIC 3220, Australia

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ABSTRACT

High pressure processing was investigated as a means to inactivate avian viral pathogens in chicken meat homogenate. Preliminary studies were conducted on eight viruses: avian influenza virus (AIV), Newcastle disease virus (NDV) and six strains of infectious bursal disease virus (IBDV). Application of 600 MPa at room temperature for up to 2 min resulted in substantial decline of virus infectivity of all strains of IBDV, AIV and NDV in chicken meat homogenate. An inactivation kinetic of IBDV-Tasik94, a very virulent strain of IBDV, showed 5 to 6 log₁₀ reduction of 50% egg infectious doses (EID₅₀)/0.2 ml in chicken meat homogenate within 10–15 s treatment at 600 MPa and room temperature. However, when measured in eggs, around 0.5 to 2.0 log₁₀ EID₅₀/0.2 ml of infectious virus remained, even after longer treatment times of up to 2.5 min. The inactivation curve was fitted to different kinetic models and a Log-decay model described best the rapid initial decline of infectivity followed by a persistent “tail” of resistant IBDV-Tasik94. Multiple pressure cycles at 600 MPa were not able to further reduce the titre of IBDV in chicken meat homogenate; however, these resistant IBDV particles were not infectious to chickens when challenged via the mucosal route. This study could inform policy on risk assessment of the importation of chicken meat products.

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1. Introduction

Infectious agents are major potential causes of loss in the poultry industries around the world, causing problems ranging from chronic insidious disease that may lead to production losses to devastating outbreaks that cause major morbidity and mortality; major losses may occur through destocking of healthy birds during stamp-out operations. Some chicken pathogens cause serious disease in humans, for example, H5N1 influenza viruses. In order to limit the losses caused by these pathogens, rigorous biocontainment is necessary to prevent their spread. One avenue of containment is the inactivation of pathogens within poultry carcasses and products. The reliable and safe inactivation of these pathogens will allow greater freedom of movement and trade of products without jeopardizing poultry and human health. In this study we examined the use of high pressure processing (HPP) to inactivate three viral

pathogens with potential to cause devastating epidemics in chickens: infectious bursal disease virus (IBDV), avian influenza virus (AIV) and Newcastle disease virus (NDV).

IBDV causes an acute, highly contagious infection of young chickens, with a predilection for the lymphoid system, particularly the bursa of Fabricius (Etteradossi & Saif, 2008). This double-stranded RNA, non-enveloped virus of the family Birnaviridae is unusually resistant to inactivation, including chemical and heat treatments (Alexander & Chettle, 1998). For this reason it persists in the environment for long periods, making it problematic to control in affected premises. The influenza viruses, of the family Orthomyxoviridae and genus Influenza virus A, infect a large range of avian and mammalian species, with high potential for species cross-over and genetic recombination between different viral types. This feature, with high inherent mutation rates, is of particular concern as it leads to the generation of novel types for which animal populations (including humans) have little immunity (Neumann & Kawaoka, 2011). In chickens, avian influenza viruses have broad tissue tropism, including for muscle tissues, and cause an acute disease that varies from unapparent to severe (Swayne &

* Corresponding author. CSIRO, Australian Animal Health Laboratory, 5 Portarlington Road, Geelong, VIC 3220, Australia.

E-mail address: John.Bingham@csiro.au (J. Bingham).

Halvorson, 2008). Newcastle disease is an acute infection of birds, the causative virus being classified under the type avian paramyxovirus 1 (APMV-1, family Paramyxoviridae). The avian paramyxoviruses infect numerous avian species causing infections with highly variable clinical outcomes (Alexander & Senne, 2008). Highly pathogenic variants of chickens have broad tissue tropism, including for respiratory, neural and intestinal tissues (Alexander & Senne, 2008).

All three viruses are transmitted by direct contact, either through inhalation or ingestion (Alexander & Senne, 2008; Etteradossi & Saif, 2008). Some influenza viruses, for instance Eurasian H5N1, are present in high amounts in muscle tissue (Bingham et al., 2009). Spread of virus through movement of poultry products, either in tissue supporting viral replication or through contamination with tissue for which the virus has a predilection, is therefore possible and may lead to disease through inadvertent consumption of those products.

High pressure processing (HPP) of foods is gaining popularity in the food industry, in part because of its ability to inactivate microorganisms and enzymes at room temperature, whilst valuable low molecular food constituents, such as vitamins, volatiles and colour pigments, remain largely unaffected. Over the last decades equipment development has progressed rapidly and HPP food products, including meat and fish products, are now available in many countries (Heinz & Buckow, 2010). HPP can effectively eliminate pathogens and prolong the shelf life of raw chicken meat (Kruk et al., 2011). However, the pressure labile nature of some meat protein systems, such as myosin or myoglobin, often limits the range of attractive commercial applications (Buckow, Sikes, & Tume, 2013).

In commercial settings, high pressure preservation of foodstuff typically involves application of static pressure not exceeding 600 MPa for a few seconds or up to several minutes. Under these conditions, many viruses can also be inactivated (Kovac, Diez-Valcarce, Hernandez, Raspor, & Rodríguez-Lázaro, 2010) possibly due to denaturation of proteins essential for host cell attachment to initiate infection (Heinz & Buckow, 2010).

The virus sensitivity to pressure, and thus extent of inactivation, is dependent on a number of environmental (e.g. pH, water activity) and processing factors, for example: pressure holding time and treatment temperature (Kingsley, 2013). Reportedly, process temperatures above and below room temperature can promote the interaction of non-polar protein side chains to water, and thus denaturation of proteins and viruses by pressure (Buckow, Isbarn, Knorr, Heinz, & Lehmacher, 2008; Knorr, Heinz, & Buckow, 2006).

At room temperature, AIV H7N7 is rapidly inactivated at pressures above 400 MPa (Isbarn, Buckow, Himmelreich, Lehmacher, & Heinz, 2007). For example, 1 min treatment at 450 MPa and 25 °C results in 7 log₁₀ reduction of the virus titre in chicken meat homogenate. Increasing process temperature has synergistic effects with pressure on the inactivation rate, and at ambient pressure the virus inactivates at 50 °C (Isbarn et al., 2007). Tian, Ruan, Qian, Shao, & Balny (2000) evaluated the effect of high pressure on IBDV strain LH in 0.9% NaCl solution using pressures of up to 240 MPa at 0 °C (initial temperature) for periods of up to 200 min. HPP (230 MPa for 1 h) of IBDV resulted in a 5 log₁₀ reduction of the virus titre (50% tissue culture infectious dose (TCID₅₀)) and chickens subsequently challenged with infectious IBDV did not develop disease (Tian, Qian, Shao, & Ruan, 1999). The treatment also resulted in morphological changes of the virus and dissociation into its subunits. Currently, there are no reports on the efficacy of high pressure to inactivate NDV.

The objective of this work was to quantify the effect of HPP at 600 MPa on the inactivation of important chicken pathogens in minced, raw chicken meat homogenate. Preliminary trials were

performed on AIV, NDV and several strains of IBDV, and more in-depth studies on one strain of IBDV. Our findings can be useful for the application of HPP to the limitation of pathogen spread via chicken meat products, specifically homogenate, and can inform risk assessment policy on importation of these products.

2. Materials and methods

All chickens and chicken eggs used in these studies were specific pathogen free (SPF), obtained from SPAFAS Australia Pty Ltd (Woodend, Victoria, Australia). All procedures conducted on chickens were approved by the CSIRO AAHL Animal Ethics Committee.

2.1. Experimental procedures

The first objective of this study was to look at the effects of HPP on a range of chicken viruses. The following eight chicken pathogens were tested in meat-virus homogenate preparations (using 9 parts meat and 1 part buffer): avian influenza virus (AIV; strain A/chicken/Victoria/1/1985 (H7N7)), Newcastle disease virus (NDV, Herts strain) and IBDV strains Tasik94 (Rudd, Heine, Sapats, Parede, & Ignjatovic, 2002), CS88, variant E, GLS-5, Aphis and 52–70. The samples were prepared and treated at 600 MPa at room temperature for a variety of treatment times, as indicated in Table 1. For each pathogen the virus titre in sub-samples of divided meat-virus homogenate was determined; from each batch one sub-sample was left untreated and the remainder were pressure treated for varying times.

Following the initial trials, detailed studies to determine inactivation rates at 600 MPa were conducted on IBDV-Tasik94 (which showed relatively high pressure resistance), mixed into chicken meat homogenate. Treatments with a single pressure cycle at 600 MPa for a variety of treatment times were performed using the same batch of IBDV-Tasik94 in chicken meat homogenate.

In a further series of experiments, the effect of multiple pressure applications was tested. In each trial there were five sub-samples of the same meat-virus mixture: three were treated by five consecutive pressure applications (each of 1 or 30 s) at 600 MPa, one was retained as an untreated control and one was treated by a single continuous pressure application of a time equal to the sum of the

Table 1

Reduction in titre of various avian viruses in chicken meat homogenate after treatment at 600 MPa and room temperature.

Virus	Pressure holding time (s)	Titration method	Start titres	End titres
			(log ₁₀ EID ₅₀ /0.2 ml)	
AIV (H7N7 Bendigo)	60	AS ^a	6.5	0
NDV (Herts)	120	AS	8.3	0.9
IBDV (Tasik94)	120	CAM ^b	5.7	1.7
IBDV (CS88)	120	CAM	4.5	0
IBDV (Aphis)	120	CAM	4.3	0
IBDV (52/70)	120	CAM	3.2	0
IBDV (variant E)	15	Chick	2.1	0
IBDV (variant E)	120	Chick ^c	≤2.7	0
IBDV (GLS-5)	15	Chick	2.5	0
IBDV (GLS-5)	120	Chick	≤2.8	0

^a AS – allantoic sac inoculation followed by haemagglutinating effect of allantoic fluid after embryo death or 5 days incubation.

^b CAM – chorioallantoic membrane inoculation followed by antigen capture ELISA for IBDV on CAM after death of embryo or 7 days after inoculation.

^c Chick – inoculation onto the mucous membranes of 3 week-old chicks and histological analysis of bursa at 3 days following inoculation.

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