CHAPTER 1 INTRODUCTION

1.1 Background

Avian cocciosis is an intestinal infection caused by obligate intracellular protozoan parasites belonging to various species of genus *Eimeria* (Lillehoj and Lillehoj, 2000). *Eimeria* enters the host by penetrating of epithelial cells of the intestinal mucosa often causing serious damage to the gut (Trout *et al.*, 1996) leading to diarrhea, dehydration, weight loss, dysentery, serious clinical illness and mortality (Cook, 1988). This disease causes impair growth rate in broilers and reduces egg production in laying hens (Mcdougald 2003; Lillehoj *et al.* 2004), and is a worldwide problem resulting in annual economic losses to the world's poultry industry estimated at 3 billion USD (Dalloul and Lillehoj, 2006).

Current methods for the control of coccidiosis are (i) incorporation of anticoccidial agents into feed or water, and (ii) use of live vaccines (Li *et al.*, 2005). However, *Eimeria* resistance to drug employed is an enormous obstacle providing that the chemical residues derived from drugs used by the poultry industry are undesirable to consumers (Li *et al.*, 2004). Further, vaccines are costly to produce, and induce species specific immunity; therefore a combination of antigens may be required to provide efficient protective immunity. In addition, there are some concerns such as vaccine safety, short shelf-life and large-scale production when live vaccines are employed (Innes *et al.*, 2006). As life cycle of *Eimeria* is associated with complex host immune response to the parasites, vaccine development has been difficult (Yun *et al.*, 2000). Therefore, there is a pressing need for new alternatives to control chicken coccidiosis.

Oral administration of specific antibodies to provide passive immunity against various pathogens in humans and animals is an attractive approach (Carlander *et al.*, 2000). The chicken egg yolk antibodies (IgY) have been applied successfully for prophylactic purposes (Lemamy *et al.*, 1999) and veterinarian therapy (Amaral *et al.*, 2002). Oral administration of IgY has been proven to be successful for the treatment against a variety of gastrointestinal pathogens such as bovine and human rotaviruses, bovine coronavirus, enterotoxigenic *Escherichia coli* and *Salmonella* spp. (Karlsson *et al.*, 2004). Using laying hens for the production of large quantities of specific antibodies is a

cost-efficient method (Carlander *et al.*, 2000). During the past 20 years, the use of chickens rather than mammals for polyclonal antibody production has increased. A major advantage of using chickens is that the antibodies can be harvested from the egg yolk instead of serum. In addition, the antibody productivity of an egg-laying hen is much higher than that of a similar sized mammal (Hau *et al.*, 2005). IgY can be produced on large scale because a hen usually lays about 280 eggs per year given that an egg yolk contains approximately 100-150 mg of IgY antibodies resulting in 28 to 42 grams of IgY yield per year per hen (Rose *et al.*, 1974). Since eggs are normal dietary components; thus, there is practically no risk of toxic side effects of IgY (Carlander *et al.*, 2000). Animal cell culture laboratory at King mongkut's university of technology thonburi has produced anti-chicken coccidia IgY and this IgY has been tested to be able to inhibit the coccidia disease progression in infected chickens. This IgY has shown potential for commercially used. Thus, large scale production of this product is the further step towards this goal. In addition, this IgY should be characterized for strategic design when applied to the farm chickens.

1.2 Objectives

1.2.1 Large scale production of anti-chicken coccidia IgY

1.2.2 Characterization of the produced IgY for the purpose of animal feed supplement

1.3 Research Outline

1.3.1 Immunization of chicken coccidia antigen into egg-laying hens at large scale

1.3.2 Detection of anti-chicken coccidia IgY

1.3.2.1 Development of ELISA method for anti-chicken coccidia IgY detection

1.3.3 Determination of immunization efficiency and the IgY production period

1.3.4 Characterization of anti-chicken coccidia IgY from egg yolk

1.3.4.1 Specificity of the IgY to *Eimeria spp.* using immunohistochemistry

1.3.5 Preparation and characterization of dried anti-chicken coccidia IgY product

1.3.5.1 Spray drying

1.3.5.2 Particle size analysis

1.3.5.3 Shelf life determination