

TABLE OF CONTENTS

LIST OF ABBREVIATIONS.....	4
1 INTRODUCTION.....	7
1. 1 Swine influenza virus.....	7
1. 1. 1 Historical overview.....	7
1. 1. 2 Clinical disease in pigs.....	8
1. 1. 3 Structure and classification.....	9
1. 1. 4 Virus transmission.....	10
1. 1. 5 Prevention and treatment.....	11
1. 1. 6 Immune response.....	12
1. 2 Probiotics.....	14
1. 2. 1 Overview of probiotics.....	14
1. 2. 2 <i>Enterococcus (E.) faecium</i> as a probiotic.....	15
1. 2. 3 Probiotics in virus infections.....	15
1. 2. 4 Mechanisms of probiotics in virus infections.....	16
1. 2. 5 Probiotics and influenza virus infection.....	17
1. 3 Zinc.....	19
1. 3. 1 Overview of zinc.....	19
1. 3. 2 Zinc oxide.....	20
1. 3. 3 Zinc in virus infections.....	20
1. 3. 4 Role of zinc in virus infections.....	21
1. 3. 5 Zinc and influenza virus infection.....	22
1. 4 Aim of study.....	23
2 MATERIALS AND METHODS.....	25
2.1 <i>In vitro</i> studies.....	25
2. 1. 1 Virus.....	25
2. 1. 2 Cell lines.....	25
2. 1. 3 Probiotic <i>E. faecium</i>	25
2. 1. 4 Experimental design of interference experiments.....	26

2. 1. 5 Cell culture	27
2. 1. 6 Virus propagation	28
2. 1. 7 MTT assay	28
2. 1. 8 TCID ₅₀ assay	28
2. 1. 9 Assessment of nitric oxide (NO) release	29
2. 1. 10 RNA extraction	29
2. 1. 11 cDNA synthesis	30
2. 1. 12 Real-time PCR to assess the expression of immune mediators	30
2. 1. 13 Virus adsorption to <i>E. faecium</i> (preincubation assay)	32
2. 1. 14 Electron microscopy	33
2. 1. 15 Statistical analysis	33
2. 2 <i>in vivo</i> studies	34
2. 2. 1 Virus	34
2. 2. 2 Vaccine	34
2. 2. 3 Feed additives	34
2. 2. 4 Animals and experimental setup	34
2. 2. 5 Clinical follow-up and sampling	36
2. 2. 6 Pathology	37
2. 2. 7 Antibody ELISA	37
2. 2. 8 Hemagglutination inhibition (HI) assay	38
2. 2. 9 Viral RNA quantification from swabs	38
2. 2. 10 Differential cell count	38
2. 2. 11 Flow cytometry	39
2. 2. 12 Statistical analysis	40
3 RESULTS	41
3. 1 <i>in vitro</i> studies	41
3. 1. 1 Cells	41
3. 1. 2 Virus propagation	41
3. 1. 3 Cytotoxicity effect of <i>E. faecium</i> on 3D4/21 and MDBK cells	42
3. 1. 4 Effect of <i>E. faecium</i> on SIV infected cells as detected by MTT assay	44

3. 1. 5 Virus titer reductions in cells treated with <i>E. faecium</i>	45
3. 1. 6 <i>E. faecium</i> increases the production of NO	46
3. 1. 7 Virus adsorption by <i>E. faecium</i>	47
3. 1. 8 Cytokine expression under the influence of <i>E. faecium</i>	48
3. 2 <i>in vivo</i> studies	50
3. 2. 1 Clinical symptoms and weight gains	50
3. 2. 2 Gross Pathology	52
3. 3. 3 Histopathology and lesion score by HE staining	53
3. 2. 4 Virological analysis	54
3. 2. 5 Antibody ELISA	55
3. 2. 6 HI assay	56
3. 2. 7 Differential cell count	57
3. 2. 8 Cellular immune responses	58
4 DISCUSSION	63
4. 1 <i>In vitro</i> study	63
4. 2 <i>in vivo</i> study	68
5 SUMMARY	73
6 ZUSAMMENFASSUNG	75
7 REFERENCES	79
PUBLICATIONS	99
ACKNOWLEDGMENTS	101