



**ENHANCING DUCK WELL-BEING THROUGH EARLY-AGE
STIMULATION, HUMAN-ANIMAL INTERACTION, AND PROBIOTIC
SUPPLEMENTATION**

By

HELEN ANAK MITIN

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfilment of the Requirements for the Degree of Doctor of Philosophy**

January 2024

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January 2024

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Intensive modern poultry production exposes birds to a multitude of stressors, posing substantial challenges to their welfare. However, there is still a lack of research on the effects of positive human-animal interaction, early-life stress, and a probiotic-supplemented diet on stress resilience, fearfulness, and growth performance in ducks undergoing processes like catching, crating, and transportation. This thesis aims to fill this gap. Hence, the thesis's first objective was to investigate the effects of positive human contact on fear and physiological stress in Pekin ducks (*Anas platyrhynchos domesticus*) subjected to crating and transport. This study exposed the ducks to human contact across various age groups. At D 42, serum levels of corticosterone (CORT), heat shock protein 70 (HSP70), and tonic immobility (TI) tests were analysed following 3 hours of crating or 3 hours of transport stresses. The study reveals that ducks that received human contact from D1 to D42 and from D1 to D21 had significantly higher levels of HSP70 expression than the no-contact group, but human contact had no effect on growth performance.

Human contact and stressors significantly affected the TI duration. The second objective aimed to determine the effects of early-life stress on growth performance, fear, and physiological stress responses, as well as gut microflora composition in both Pekin and Muscovy (*Cairina moschata*) ducks subjected to transportation stress at market age. The ducks were stressed at D3 to D14 by keeping them in a climate chamber set at 24°C±1 and hearing 100 dB of noise (construction and road traffic noises) for three hours every day, and at D42, the ducks underwent three hours of transportation stress. Blood analyses for CORT and HSP70 were done at D14 and D42. The RT-PCR for the gut microbiota count and TI tests were conducted at D42. The results showed that subjecting Pekin ducks to early-life stress can alleviate the stress associated with transportation at market age by significantly changing the CORT and HSP70 levels in Pekin ducks but not in Muscovy ducks. The TI test demonstrated a significant breed difference, with Muscovy ducks exhibiting a longer TI duration and more attempts compared to Pekin ducks. The Pekin ducks showed significantly higher weight gains and feed intake compared to Muscovy ducks. Early-life stress is also beneficial in maintaining the gut population of *Lactobacillus spp.* following transportation.

For the third and fourth objectives, the experiments were conducted on Pekin ducks that were fed with probiotic-supplemented feed containing CLOSTAT® (*Bacillus subtilis*) at a concentration of 1 g/kg on D1 to D21. Blood tests for heterophil-to-lymphocyte ratio (HLR), serum biochemistry such as creatine kinase (CK), total protein (TP), triglycerides (TG), glucose (GLU), total cholesterol (CHOL), and lactate (LACT), stress hormonal (CORT and HSP70), TI, and exploratory behaviour tests were determined at D21 after 4 hours of crating stress.

The findings revealed that the dietary intervention did not exert a significant impact on growth performance. Despite this, the probiotic-added diet significantly reduced the levels of CORT, HSP70, HLR, TG, and CK in ducks compared to those fed the control diet. The exploratory and fear-related behaviour tests also showed that *B. subtilis* supplementation significantly decreased TI duration, lowered TI attempts, and increased their exploratory activity in a new environment. In summary, the experiments indicate that positive human contact, early life stress, and dietary probiotic supplementation collectively have a significant impact on stress resilience in ducks, but they did not affect growth performance.

Keywords: behaviour, duck, fear, human-animal interaction, probiotic, stress.

SDG: GOAL 1: End poverty, GOAL 2: End hunger, GOAL 12: Responsible consumption and production

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**MENINGKATKAN KESEJAHTERAAN ITIK MELALUI RANGSANGAN
AWAL USIA, INTERAKSI MANUSIA-HAIWAN DAN SUPLEMEN
PROBIOTIK**

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Sistem pengeluaran unggas yang intensif mendedahkan unggas kepada pelbagai stress yang boleh menjejaskan kebajikan mereka. Namun begitu, masih ada kekurangan penyelidikan mengenai kesan interaksi manusia-haiwan yang positif, stres awal hayat, dan diet tambahan probiotik terhadap daya tahan stres, ketakutan, dan prestasi pertumbuhan itik dalam menghadapi proses penangkapan, kekangan dan pengangkutan. Tesis ini bertujuan untuk menerokai perkara ini. Oleh itu, objektif pertama tesis adalah untuk menyiasat kesan sentuhan manusia positif terhadap ketakutan dan stres fisiologi dalam itik Pekin (*Anas platyrhynchos domesticus*) terhadap kekangan dan pengangkutan. Kajian ini mendedahkan itik kepada sentuhan manusia di pelbagai peringkat umur. Pada D 42, tahap serum kortikosteron (CORT), protein kejutan haba 70 (HSP70), dan ujian imobiliti tonik (TI) dianalisa selepas 3 jam kekangan atau 3 jam pengangkutan.

Kajian itu mendapati bahawa itik yang menerima sentuhan manusia dari D1 hingga D42 dan dari D1 hingga D21 mempunyai tahap ekspresi HSP70 yang jauh lebih tinggi daripada kumpulan tanpa sentuhan manusia, tetapi sentuhan manusia tidak mempunyai kesan ke atas prestasi tumbesaran itik. Sentuhan manusia dan stres ini didapati mempengaruhi tempoh TI yang ketara. Objektif kedua bertujuan untuk menentukan kesan stres awal hayat terhadap prestasi tumbesaran, ketakutan, dan tindak balas tekanan fisiologi, serta komposisi mikroflora usus pada itik Pekin dan Muscovy (*Cairina moschata*) setelah mengalami stres pengangkutan pada usia pasaran. Dari D3 hingga D14 anak itik diletakan di dalam bilik pada suhu $24^{\circ}\text{C} \pm 1$ dan didedahkan kepada 100 dB bunyi (bunyi kawasan pembinaan dan lalu lintas jalan) selama tiga jam setiap hari, dan pada D42, itik diangkut dengan lori selama tiga jam. Analisis darah untuk CORT dan HSP70 dilakukan pada D14 dan D42. Ujian RT-PCR untuk kiraan mikrobiota usus dan ujian TI telah dijalankan di D42. Keputusan menunjukkan bahawa stress awal hayat boleh mengurangkan tekanan yang berkaitan dengan pengangkutan pada usia pasaran dengan mengubah paras CORT dan HSP70 dengan ketara dalam itik Pekin tetapi tidak dalam itik Muscovy. Ujian TI menunjukkan perbezaan baka yang ketara, dengan itik Muscovy mempamerkan tempoh TI yang lebih lama dan lebih banyak percubaan berbanding itik Pekin. Itik Pekin menunjukkan pertambahan berat badan dan pengambilan makanan yang jauh lebih tinggi berbanding itik Muscovy. Tekanan awal hayat juga bermanfaat dalam mengekalkan populasi usus *Lactobacillus spp.* berikutan stres pengangkutan. Untuk objektif ketiga dan keempat, eksperimen dijalankan ke atas itik Pekin yang diberi makanan tambahan probiotik yang mengandungi CLOSTAT[®] (*Bacillus subtilis*) pada kepekatan 1 g/kg pada D1 hingga D21.

Ujian darah untuk nisbah heterofil-kepada-limfosit (HLR), biokimia serum seperti creatine kinase (CK), protein total (TP), trigliserida (TG), glukosa (GLU), kolesterol total (CHOL), dan laktat (LACT) , hormon tekanan (CORT dan HSP70), TI, dan ujian tingkah laku penerokaan ditentukan pada D21 selepas 4 jam stres kekangan. Penemuan menunjukkan penambahan probiotik di dalam makanan tidak memberi kesan terhadap prestasi tumbesaran itik. Walaupun begitu, penambahan probiotik di dalam makanan itik telah mengurangkan tahap CORT, HSP70, HLR, TG, dan CK dalam itik dengan ketara berbanding mereka yang diberi diet kawalan selepas stress kekangan. Ujian penerokaan dan tingkah laku berkaitan ketakutan juga menunjukkan bahawa suplemen *B. subtilis* mengurangkan tempoh TI dengan ketara, merendahkan percubaan TI dan meningkatkan aktiviti penerokaan mereka dalam persekitaran baharu. Ringkasnya, eksperimen menunjukkan bahawa hubungan manusia yang positif, tekanan hidup awal, dan suplemen probiotik diet secara kolektif mempunyai kesan yang signifikan terhadap daya tahan tekanan dalam itik, tetapi ia tidak menjejaskan prestasi pertumbuhan.

Kata Kunci: itik, interaksi manusia-haiwan, ketakutan, tingkahlaku, stres

SDG: MATLAMAT 1: Tiada Kemiskinan, MATLAMAT 2: Sifar Kebuluran, MATLAMAT 12: Penggunaan dan Pengeluaran Bertanggungjawab

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TABLE OF CONTENTS

	Page
ABSTRACT	i
ABSTRAK	iv
ACKNOWLEDGEMENTS	vii
APPROVAL	viii
DECLARATION	x
LIST OF TABLES	xv
LIST OF FIGURES	xviii
LIST OF DIAGRAMS	xix
LIST OF ABBREVIATIONS	xx
 CHAPTER	
 1 INTRODUCTION	 1
1.1 The Objectives	5
1.2 The Hypotheses	6
 2 LITERATURE REVIEW	 7
2.1 Duck Production in Malaysia	7
2.2 Human-Animal Relationship	8
2.3 Early-Life Stress and Welfare	10
2.4 Animal Production and Growth Performance Responses to Stress	13
2.5 Animal Physiological Responses to Fear and Stress	14
2.5.1 Hormonal Responses	15
2.5.2 Hematological and Biochemistry Responses	19
2.5.3 Gut-Microbiota Population Responses to Stress	21
2.6 Animal Behaviour Responses to Stress	25
2.6.1 Fear Behaviour	27
2.6.2 Exploratory Behaviour	28
2.7 Effects of Probiotic Supplementation on Growth Performance and Stress Behaviour in Animals	33
2.7.1 Animal Growth Performance	33
2.7.2 Animal Behaviours	37
 3 EFFECTS OF POSITIVE HUMAN CONTACT ON FEAR AND PHYSIOLOGICAL STRESS IN PEKIN DUCKS (<i>Anas platyrhynchos domesticus</i>) SUBJECTED TO CRATING AND TRANSPORT	 42
3.1 Introduction	42
3.2 Materials and Methods	44
3.2.1 Animals and Housing	44
3.2.2 Experimental Design and Treatment Groups	45
3.2.3 Growth Performance and Mortality	46
3.2.4 Crating	47
3.2.5 Road Transportation	47

3.2.6	Tonic Immobility Test	47
3.2.7	Blood Tests	48
3.3	Statistical Analysis	49
3.4	Results	49
3.4.1	Growth Performance	49
3.4.2	Tonic Immobility Test	50
3.4.3	Serum Corticosterone Level	51
3.4.4	Serum Level of HSP70	52
3.5	Discussion	53
3.6	Conclusions	57
4	EFFECTS OF EARLY LIFE STRESS ON GROWTH PERFORMANCE, FEAR AND PHYSIOLOGICAL STRESS RESPONSES, AND GUT MICROFLORA IN PEKIN (<i>Anas platyrhynchos domestica</i>) AND MUSCOVY (<i>Cairina moschata</i>) DUCKS SUBJECTED TO TRANSPORTATION	59
4.1	Introduction	59
4.2	Material and Methods	61
4.2.1	Animals and Housing	62
4.2.2	Experimental Design and Treatment Groups	62
4.2.3	Early Stress Treatment	64
4.2.4	Road Transportation	64
4.2.5	Growth Performance	65
4.2.6	Blood Sampling	65
4.2.7	Tonic Immobility Test	65
4.2.8	Determination of Circulating Corticosterone and Heat Shock Protein 70	66
4.2.9	Caecal Bacterial Quantification	67
4.2.10	Quantitative Real Time PCR	67
4.3	Statistical Analysis	68
4.4	Results	69
4.4.1	Body Weight, Feed Intake and Feed Conversion Ratio (FCR)	69
4.4.2	Tonic Immobility Test	69
4.4.3	Serum Corticosterone Level	71
4.4.4	Serum Level of Heat Shock Protein 70	74
4.4.5	Microbiota Population	75
4.5	Discussion	76
4.6	Conclusions	82
5	EFFECT OF <i>Bacillus subtilis</i> SUPPLEMENTATION ON GROWTH PERFORMANCE, HAEMATOLOGY, SERUM BIOCHEMISTRY AND HORMONAL RESPONSES IN STRESSED PEKIN DUCKS (<i>Anas platyrhynchos domestica</i>).	83
5.1	Introduction	83
5.2	Materials and Methods	85
5.2.1	Animals and Housing	85
5.2.2	Experimental Design and Treatment Groups	86
5.2.3	Growth Performance and Mortality	87
5.2.4	Blood Sampling	88

5.2.5	Measurement of Blood Parameters	89
5.3	Statistical Analysis	90
5.4	Results	91
5.4.1	Body Weight, Feed Intake and Feed Conversion Ratio (FCR)	91
5.4.2	Serum Corticosterone and Heat Shock Protein 70 Levels, and Heterophil to Lymphocyte Ratios	91
5.4.3	Blood Biochemistry Tests	92
5.5	Discussion	93
5.6	Conclusions	98
6	FEAR-RELATED BEHAVIOUR AS AFFECTED BY <i>Bacillus subtilis</i> SUPPLEMENTATION IN PEKIN DUCKS (<i>Anas platyrhynchos domesticus</i>).	99
6.1	Introduction	99
6.2	Materials and Methods	101
6.2.1	Animals, Housing, Husbandry and Experimental Design	102
6.2.2	Tonic Immobility Test	102
6.2.3	Behaviour Tests	103
6.3	Statistical Analysis	109
6.4	Results	110
6.4.1	Tonic Immobility Test	110
6.4.2	Open-Field Test (OFT)	110
6.4.3	Pen and Cone Test (PCT)	112
6.4.4	Response to the Presence of Man Test (RMT)	112
6.4.5	Novel Arena Test (NAT)	113
6.5	Discussion	114
6.6	Conclusions	118
7	GENERAL CONCLUSION AND RECOMMENDATION FOR FUTURE RESEARCH	119
7.1	General Conclusion	119
7.2	Recommendations	122
	REFERENCES	125
	APPENDICES	164
	BIODATA OF STUDENT	165
	LIST OF PUBLICATIONS	167

LIST OF TABLES

Table	Page
2.1 Duck population in Malaysia	8
2.2 Supply and utilization of duck meat in Malaysia	8
3.1 Mean ($\pm$ S.E.M) body weight, feed intake, FCR and mortality rates by human contact treatment	50
3.2 Mean ($\pm$ S.E.M) duration of tonic immobility (TI, s) and number of attempts to induce TI by human contact treatment and sampling point	51
3.3 Mean ($\pm$ S.E.M) tonic immobility durations where human contact treatment x sampling point interactions were significant	51
3.4 Mean ($\pm$ S.E.M) serum corticosterone (CORT) (ng/ml) and serum level of heat shock protein 70 levels (ng/mL) by human contact treatment and sampling point	52
3.5 Mean ($\pm$ S.E.M) serum corticosterone concentrations (ng/mL) where human contact treatment x sampling point interactions were significant	53
3.6 Mean ($\pm$ SEM) serum levels of heat shock protein 70 (ng/mL) where human contact treatment x sampling point interactions were significant	53
4.1 Primers used for the determination of the microbial population of ducks cecal digesta	68
4.2 Effect of breed and early stress treatment on the body weight, feed intake and FCR in ducks from days 1 to 42 (mean+SEM)	70
4.3 Effect of breed and the combination of early and late stress treatment on tonic immobility (TI) duration and the number of TI inductions in ducks on day 42 (mean +SEM)	70
4.4 Effect of breed and early stress treatment on serum corticosterone levels (CORT) in ducks on day 14 (mean +SEM)	71
4.5 Mean (+SEM) serum corticosterone levels (ng/mL) where breed x early stress treatment interactions were significant on day 14	72
4.6 Effect of breed and the combination of early and late stress treatment on serum corticosterone levels (CORT) in ducks on day 42 (mean+SEM)	73

4.7	Mean ($\pm$ S.E.M) serum corticosterone levels (ng/mL) at D 42 where breed x stress treatment interactions were significant	73
4.8	Effect of breed and early stress treatment on serum heat shock protein (HSP) 70 levels on day 14 (mean +SEM)	74
4.9	Effect of breed and combination of early and late stress treatment on serum heat shock protein (HSP) 70 levels in ducks on day 42 (mean +SEM)	75
4.10	Effect of breed and the combination of early and late stress treatment on caecal bacterial counts ($\log_{10}$ cfu/g) in ducks on day 42 (mean +SEM)	76
5.1	Mean ($\pm$ S.E.M) weight gains, feed intake, feed conversion ratios (FCR) by diet from D 1-21	91
5.2	Mean ($\pm$ S.E.M) serum corticosterone (CORT) (ng/mL) and heat shock protein (HSP) 70 (ng/mL) levels, and heterophil to lymphocyte ratios (HLR) by diet and crating treatment on D 21	92
5.3	Mean ($\pm$ S.E.M) serum corticosterone levels (ng/mL) at D 21 where diet x crating treatment interactions were significant	92
5.4	Mean ($\pm$ S.E.M) serum levels of creatinine kinase (CK) (mg/mL), total protein (TP) (g/mL), triglyceride (TG) (mg/mL), glucose (GLU) (mg/mL), cholesterol (CHOL) (mg/mL), and lactate (LAC) (mg/mL) by diet and crating treatment on D 21	93
6.1	Mean ($\pm$ S.E.M) tonic immobility duration (s) and number of attempts by diet and crating treatment on D 21	110
6.2	Mean ($\pm$ S.E.M) duration of latency to the first immobilisation (LA) (s), duration of mobility (DM) (s), number of zones visited (ZV), number of pecks directed to the floor or the walls (P), frequency of body shaking (BS), and number of vocalisation (V) in open-field test	111
6.3	Mean ($\pm$ S.E.M) duration of latency to the first immobilisation (LA) (s) and frequency of body shaking (BS) where diet x crating treatment interactions were significant	111
6.4	Mean ($\pm$ S.E.M) of PCT total score in pen and cone test	112
6.5	Mean ($\pm$ S.E.M) duration of latency to the first immobilisation (LA, s), duration of mobility (DM, s), number of pecks directed to the floor or the walls (P), frequency of body shaking (BS), and number of vocalisation (V), and avoidance index (AI) in response to the presence of men test	113

- 6.6 Mean ($\pm$ S.E.M) vocalisation (V, no. of peep), number of squares visited (SV, 0 to 3), number of escape from the arena (ES), duration of latency to ambulate (LM, s), duration of duck activity in the in pen doing walking or running (AVA, s), duration of foraging or pecks/searching for food (FR, s) for novel arena test

114



LIST OF FIGURES

Figure	Page
6.1 Illustration of the open-field test arena	104
6.2 Experimental set-up for open-field test – video recording view	104
6.3 Illustration of the response to the presence of men test arena	105
6.4 Experimental set-up for response to the presence of men test- video recording view	106
6.5 Illustration of the Pen and cone test	107
6.6 Experimental set-up for Pen with cone test- video recording view	108
6.7 Experimental set-up for Novel Arena test (Video recording view)	109

LIST OF DIAGRAMS

Diagram	Page
3.1 Experimental design for Chapter 3	46
4.1 Experimental design for Chapter 4	63
5.1 Experimental design for Chapter 5 and Chapter 6	88



LIST OF ABBREVIATIONS

CHOL	cholesterol
CK	creatine kinase
CORT	serum corticosterone level
DVS	Department of Veterinary Services, Malaysia
D	day/days
doa	day/days of age
GLU	glucose
HAR	human animal relationship
HLR	heterophil lymphocyte ratio
HSP70	heat shock protein 70
HPA	hypothalamic-pituitary-adrenal
LAC	lactate
m	Meter
min	minute/minutes
MGBA	microbiota-gut-brain-axis
mL	millilitre
NAT	novel arena test
OFT	open-field test
PCT	pen and cone test
RMT	presence of man test
rpm	revolutions per minute
s	second/seconds
TI	tonic immobility
TG	triglycerides
TP	total protein

UPM

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°C

degree Celsius



CHAPTER 1

INTRODUCTION

Advancements in genetics, nutrition, housing, and management strategies have propelled the modern poultry industry forward, fostering remarkable gains in productivity. However, this enhanced efficiency also brings birds closer to their physiological limits, heightening the significance of nutrition, environment, and management practices (Korver, 2023). In Malaysia, the duck production sector has undergone a significant evolution, transitioning from traditional backyard management to semi-intensive and finally to intensive systems. Initially, ducks were reared with basic shelter and access to open areas for movement and swimming (Azahan and Mokhtar, 1984). Over time, this approach has shifted towards more intensive methods, with ducks now housed in enclosed facilities. As per the Department of Veterinary Services Malaysia's 2021 data, the country's duck population stands at an estimated 9,678,749. Notably, in 2020, Singapore imported over 50% of Malaysia's duck population, reflecting a substantial export value of RM 124.14 million (DVS Statistic, 2020/2021).

Some of the key factors that can have a negative impact on poultry welfare if not handled carefully include human contact or handling (Acharya et al., 2022), housing systems (Abdel-Hamid et al., 2020), higher stocking density (Buijs et al., 2009; Cengiz et al., 2015), thermal stress (Zulkifli et al., 2009; Bueno et al., 2017; Chen et al., 2021b), crates (Weeks et al., 2019), and transportation (Jacobs et al., 2017; Scanes et al., 2020). Therefore, it's crucial to implement strategies aimed at enhancing poultry resilience and mitigating environmental stressors. It's widely recognised that both

acute and chronic stress can have adverse effects on welfare (Dobson et al., 2007). Researchers have extensively investigated the advantages of early-life stressors, such as thermal stress, feed restriction, and noise, in promoting stress resilience in chickens later in life (Zulkifli et al., 2002b; Zhang et al., 2009; El-Moniary et al., 2010; Flores et al., 2019). However, there is a dearth of similar research on ducks..

Given the limited research on the effects of positive human interaction, early-life stress, and probiotic supplementation in ducks undergoing processes like catching, crating, and transportation, this thesis endeavours to address this gap. Its objective is to offer valuable insights into improving the welfare and performance of ducks within intensive poultry production systems. Through an examination of the impacts of positive human contact, early-life stress, and probiotic supplementation (specifically *B. subtilis*) on duck behaviour, the research aims to provide practical strategies for reducing stress and enhancing resilience in ducks. Ultimately, the goal is to improve ducks' overall well-being and productivity in such systems.

The quality of interactions between humans and animals significantly influences the productivity and welfare of farm animals (Acharya et al., 2022). Such interactions can evoke various responses in animals, ranging from neutral to positive or negative (Zulkifli, 2013). Studies have shown that frequent positive interactions with humans can induce favourable changes in chicken physiology, behaviour, health, and productivity (Taylor et al., 2022). Conversely, negative human contact can instill significant fear in animals, potentially impairing their growth and reproductive capabilities. Researchers have observed that regular visual and physical contact with commercial broiler chicks reduces underlying fearfulness in chickens (Zulkifli, 2013;

Rault et al., 2020). Panic responses in quail, characterised by hyperexcitability and violent escape reactions, are common reactions to fear (Mills & Faure, 1991). Extensive documentation exists on efforts to modify the physiological and behavioural characteristics of livestock species, including cattle, sheep, pigs, horses, and poultry, through consistent human interaction (Hemsworth & Gonyou, 1997; Hemsworth & Barnett, 2000). Inappropriate handling techniques that induce aversive experiences in farm animals can lead to avoidance behaviour, a manifestation of fear recognised as a stress response (Rushen et al., 1999).

When it comes to duck production, one important question that emerges is the duration during which physical human interaction and handling activities are crucial. Jones and Waddington (1992) found that there is no distinct period of heightened sensitivity for regular chick handling. Zulkifli et al. (2002a) found that regular visual interaction with humans during the initial 21 days of life in broiler chickens decreased anxiety and stress reactions to handling and crate confinement while simultaneously enhancing antibody responses. Gross (1983) and Zulkifli et al. (2002a) observed that stimulation during the early stages of life, a critical time when several biological systems are undergoing development, can have long-lasting consequences and impact gene expression. However, there is presently a lack of knowledge about the impact of physical human touch or handling on young ducks.

Literature suggests that poultry breeds and strains exhibit varying responses to environmental stressors (Debut et al., 2005; Huff et al., 2007; Campo et al., 2008; Soleimani et al., 2011). Studies by Faure et al. (2003), Guémené et al. (2006), and Arnaud et al. (2008) delved into the genetic underpinnings of stress and fear reactions

in ducks and their hybrids. They observed that Pekin ducks displayed higher locomotor activity and were more fearful compared to Muscovy ducks, which exhibited a greater avoidance of humans. Interestingly, their hybrids (mule ducks) exhibited heightened panic responses and a specific fear of humans compared to the parental genotypes. Furthermore, studies have highlighted sex-specific differences in responses to environmental stressors. Elfving et al. (2015) investigated the impact of early stress on chickens (*Gallus gallus domesticus*), focusing on sex-specific effects. The researchers discovered that males who experienced stress early in life had elevated levels of anxiety in an open field test, shorter periods of tonic immobility, and a tendency towards delayed sexual maturation. This was supported by lower levels of testosterone compared to the control group. Interestingly, females showed no such effects. Therefore, considering the breed and sex-specific responses to stress, male Pekin ducklings were mainly selected as the subjects for experimentation in this study.

Probiotic supplementation in poultry feed has shown inconsistent outcomes in terms of growth performance. The competition between beneficial bacteria for attachment sites and nutrients within the intestinal tract is the ultimate objective of displacing potentially deleterious bacteria using probiotics (Harimurti and Hadisaputro, 2015). Oladokun and Adewole (2023) conducted a study on the impact of probiotic-supplemented diets in chickens, using *Bacillus subtilis* and *Bacillus licheniformis*, and found no difference in average body weight gain or feed conversion ratio (FCR) between the treatment groups at D 35. In contrast with Guo et al.'s (2016) findings that dietary supplementation with *B. subtilis* enhances growth performance in Pekin ducks, Studies on the administration of probiotics derived from *Bacillus amyloquelaciens* have shown reduced aggression and distress vocalizations among turkeys (Abdel-

Azeem et al., 2013). Parois et al. (2017) conducted a study on the effects of dietary supplementation with a probiotic (*Pediococcus acidilactici*) on female Japanese quail (*Coturnix japonica*), which revealed a decrease in emotional reactivity and an improvement in memory among the quails. Given the extensive research and viable findings on the effect of *B. subtilis* supplementation in animal feed on growth performance but a lack of studies on behaviour, this probiotic was selected for the experiment.

Considering the unexplored areas and the dearth of information regarding the effects of human contact, early stress, and probiotic supplementation on stress resilience, underlying fearfulness, and behaviour in duck production, further investigation is warranted. Therefore, the objectives and hypotheses of the study were formulated as follows:

1.1 The Objectives

Therefore, the objectives of this study were:

- 1.1.1. to investigate the effects of positive human contact on fear and physiological stress in Pekin ducks (*Anas platyrhynchos domesticus*) subjected to crating and transport.
- 1.1.2. to determine the effects of early-life stress on growth performance, fear, and stress responses, as well as gut microflora composition in both Pekin and Muscovy ducks at market age subjected to transportation stress.
- 1.1.3. To determine the effect of *Bacillus subtilis* supplementation on growth performance, heterophil and lymphocyte ratio, serum biochemistry, and hormonal responses in stressed 21-day-old Pekin ducks.

- 1.1.4. To investigate the effects of *Bacillus subtilis* supplementation on exploratory and fear-related behaviours in stressed 21-day-old Pekin ducks.

1.2 The Hypotheses

In this study, the hypotheses were:

- 1.2.1 Positive human contact can enhance growth performance and mitigate stress and fear reactions in Pekin ducks undergoing crating and transportation.
- 1.2.2 Physiological stress and fear responses, as well as gut microbiota composition, in road-transported Pekin and Muscovy ducks at market age, may be influenced by early-life stress.
- 1.2.3 Supplementing diets with *Bacillus subtilis* enhances growth performance and may alleviate the physiological stress response to catching and crating in stressed 21 day old Pekin ducks.
- 1.2.4 Supplementation of *Bacillus subtilis* in the diet can alter the exploratory and fear-related behaviour in stressed 21 day old Pekin ducks.

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